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Investigating host genetics and the role of selection for increased resistance to bovine tuberculosis in dairy cattle

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Declaration

I hereby declare that this thesis and the work presented in it are my own unless otherwise specified. The thesis is also a product of my own original research. This work has not been submitted for any other degree or professional qualification except as specified. Where I consulted published work of others, it was always clearly attributed by proper use of quotes and references. This thesis is a chronicle of my PhD studies at the University of Edinburgh.

Kethusegile Raphaka

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This thesis is dedicated to God Almighty for His love and kindness.

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Abstract

The significant social and economic losses as a result of bovine tuberculosis (bTB) present a continuous challenge to cattle industries in the United Kingdom (UK) and worldwide. Furthermore, as a zoonotic disease, bTB may pose a threat to humans. The potential transmission of bTB in cattle, estimated by the basic reproductive ratio (R_0) was found to range between 1.0 and 1.9 in previous studies. In the UK, there has been an overall increase in bTB incidence in the last two decades despite national control and eradication programmes spanning over five decades. Such programmes mainly consist of surveillance based on the administration of skin tests and culling of animals reacting positive to these tests. Animal mobility restrictions are implemented in this case. At the same time, several studies have demonstrated that there is significant host genetic variation in individual cattle susceptibility to bTB, making the disease amenable to improvement with genetic or genomic selection. In addition, genomic analyses enhance the understanding of genetic mechanisms underlying the disease and its dynamics.

The overall aim of this PhD thesis was to address existing scientific research gaps on the genetics of bTB resistance in dairy cattle. The following specific objectives were set: 1) to identify genomic regions underlying susceptibility to bTB using novel trait definitions, 2) to quantify the impact of long-term genetic selection for increased resistance to bTB on disease prevalence and dynamics and 3) to determine the consequences of genetically selecting for increased resistance to bTB on other economically important traits in dairy cattle.

Genome-wide association studies (GWAS), regional heritability mapping (RHM) and chromosomal association analyses were applied in order to identify

genomic regions associated with bTB (objective 1). Phenotypes comprised de-regressed estimated breeding values of 804 Holstein-Friesian sires obtain from the UK national genetic evaluation for bTB. Phenotypes pertained to three bTB trait definitions: i) positive reactors to the skin test with positive post-mortem examination results (phenotype 1); ii) positive reactors to the skin test regardless of post-mortem examination results (phenotype 2) and iii) as in (ii) plus non-reactors and inconclusive reactors to the skin test with positive post-mortem examination results (phenotype 3). In all cases, non-reactors without a subsequent positive post-mortem were considered to be healthy animals with regards to bTB. Genotypes based on a 50K SNP DNA array were available and a total of 34,874 SNPs remained after quality control. The estimated polygenic heritability for susceptibility to bTB was 0.26, 0.37 and 0.34 for phenotypes 1, 2 and 3, respectively. GWAS identified a putative SNP on *Bos taurus* autosomes (BTA) 2 associated with phenotype 1, and another on BTA 23 associated with phenotype 2. Genomic regions encompassing these SNPs were found to harbour potentially relevant annotated genes. RHM confirmed the effect of these genomic regions and identified new regions on BTA 18 for phenotype 1 and BTA 3 for phenotypes 2 and 3. Heritabilities of the genomic regions ranged between 0.05 and 0.08 across the three phenotypes. Chromosome association analysis indicated a major role of BTA 23 on susceptibility to bTB.

A stochastic genetic epidemiological model based on four main disease states, namely susceptible (*S*), exposed (*E*), infectious (*I*) and test-sensitive (*T*), was developed to address objective 2. Effects of selection for increased resistance to bTB were investigated in a closed, genetically heterogeneous simulated population whose structure reflected the UK national dairy herd. Disease dynamics reflected real bTB

data from the UK national genetic evaluation. The proposed genetic epidemiological model was implemented to simulate breakdowns under both absence and presence of selection. Genetic selection was simulated over 20 generations in 50 replicates, while exploring various selection intensities reflecting selection of the 10, 25, 50, 70 and 100% (no selection scenario) most resistant sires. Results indicated that selection significantly reduced the average underlying susceptibility across generations. The risk of breakdown was reduced by half after 4 and 6 generations for high selection intensities (10 or 25% of sires selected) and after 9 and 15 generations for low selection intensities (50 or 70% of sires selected). The average percentage of secondary cases was reduced to less than 1% in 4 and 5 generations for high selection intensities, and in 7 and 11 generations for low selection intensities. The reduction in the number of secondary cases across generations could also be indicative of the possible impact of genetic selection on the basic reproductive ratio (R_0) which is defined as the number of secondary cases that results from an infectious individual in a naive population. Genetic selection also reduced severity and duration of breakdowns across generations.

Finally, with regards to objective 3, a stochastic simulation was used to investigate the long-term effects of selection for resistance to bTB on other economically important traits in the UK dairy selection programme. Selection was simulated in a genetically heterogeneous population across 10 generations in 50 replicates. Animal genetic values for bTB and other traits were simulated based on variance and genetic correlation estimates obtained from literature. Independent culling levels selection of sires was applied in every generation whereby selection was first based on increasing resistance to bTB, then improving either an overall index,

milk fat yield (FY) or milk protein yield (PY). This mimics real life practices regarding the newly released national genetic evaluations for bTB resistance. The overall index comprised several traits of interest such as milk yield (MY), FY, PY, feet and legs (FL), mammary (MAM), milk somatic cell count (SCC), calving interval (CI), non-return to service at 56 days (NR56) and lifespan (LS). A fertility index (FI) consisting of CI and NR56 was also considered in the analyses. Regarding bTB, different levels of selection intensities were explored corresponding to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires. Two levels of selection intensity on the overall index, FY or PY were considered corresponding to selecting the best 5 and 10% of sires that were left after first selecting for bTB resistance. Results indicated that selection for increased bTB resistance would generally not have far-reaching consequences on other important traits. As expected, susceptibility to bTB declined with time and increasing selection intensity. Trends for all production traits (MY, FY and PY) in the present study were affected by selection for increased bTB resistance because of their significant genetic correlations with bTB. However, body conformation traits (FL and MAM) were not affected by selection for increased bTB resistance due to zero correlation assumed between these traits and bTB in the present study. Selection on bTB hampered improvement of SCC but enhanced LS because it was correlated unfavourably with SCC but favourably with LS. In all selection scenarios, the overall index improved and was generally not affected by selection for bTB resistance. Similarly, the FI was not affected by selection on bTB in all cases. However, secondary selection on production traits only (FY or PY) led to a decline in FI.

Results presented in this thesis add insight into the genetic architecture of bTB and offer a prediction of potential effects of genetic selection for increased resistance to bTB in dairy cattle. The genomic regions and candidate genes identified to be associated with susceptibility to bTB will assist to further elucidate pathways critical to cattle susceptibility to bTB. Consistent with previous studies of other populations and trait definitions, results from genomic association analyses suggest that susceptibility of cattle to bTB is heritable and likely a polygenic trait, amenable to improvement by genetic and/or genomic selection. Embarking on routine selection for resistance to bTB will reduce future bTB prevalence and severity of breakdowns across selection generations, as manifested by results of this thesis. The results also highlight the importance of considering selection as a complementary strategy to existing interventions. This has the potential to accelerate control and ultimate eradication of bTB. This strategy could assist the UK to achieve the national goal of being officially bTB free by 2038. Furthermore, as indicated by results of this thesis, selection against bTB in the national breeding programme will not adversely affect other economically important traits. Assimilation of bTB into the overall index will better manage possible antagonistic correlations between bTB susceptibility and some of the other traits.

Lay summary

Bovine tuberculosis has led to high economic losses for both farmers and governments across the world. Apart from affecting the welfare of animals, and trade on cattle and related products, it may also be a threat to humans. Bovine tuberculosis is a concern in the United Kingdom (UK) where the incidence of bTB has been increasing in the last two decades despite national control and eradication programmes spanning over five decades. However, several studies have demonstrated that there is significant variation in the susceptibility of individual animals to bTB, making it possible to genetically select for enhanced animal resistance to the disease.

Objectives of this thesis were: firstly, to identify regions in the entire cattle genome that are related to the disease with a view of finding out the genes that are potentially involved, secondly, to determine the effect of selection for resistance to bTB on future prevalence of the disease and finally, to establish the effects of selection for resistance to bTB on other important traits.

Using information from the UK national genetic evaluations for bTB and applying different methods, the first study in this thesis managed to identify regions that were suggestive of association with bTB. These regions harboured genes that have been found to have a role in immune response to human diseases and therefore may possibly play a part in bTB infection. In addressing the second objective a simulation model representing different states that an animal can be in during the course of bTB infection was developed. The model was used in conjunction with variation in individual animal susceptibility to bTB to establish the effects of selection for resistance to bTB on future prevalence of the disease. Results from this analysis showed that selection for resistance to bTB reduces both prevalence and severity of

epidemics in future generations. Finally, an analysis to establish the effects of selection for improved bTB resistance on other economically important traits was performed. Results from this analysis demonstrated that selection for bTB resistance will not negatively affect other traits in the future; instead susceptibility to bTB will decrease while other traits may be improved in future generations.

Results presented in this thesis provide an insight into the genetic characteristics of bTB and the potential role of genetic selection for increased resistance to bTB in dairy cattle. Results highlight the importance of considering selection as a complementary strategy to the current interventions. This has the potential to accelerate the control and ultimate eradication of bTB especially in countries like the UK where the vision is to achieve the national goal of being officially bTB free by 2038.

CHAPTER 1

General Introduction

1.1 Bovine tuberculosis and disease transmission

Bovine tuberculosis (bTB) is a chronic bacterial disease caused by *Mycobacterium bovis (M. bovis)* infection and usually manifests with tuberculous lesions predominantly in the respiratory tract; however, lesions could also be found elsewhere [1]. In fact, between 70 to 90% of lesions are found in either the lymph nodes of the head or the thoracic cavity [2]. While cattle are considered the primary host, *M. bovis* has a large number of additional hosts including wildlife species and humans. Respiratory excretion and inhalation is considered to be the primary route through which transmission of *M. bovis* from cattle-to-cattle occurs.

Transmission is generally facilitated by close, prolonged contact between infected and healthy animals [3, 4]. Therefore, conditions under intensive livestock production, where animals are kept closer to each other, favour the spread of *M. bovis.* The transmission of *M. bovis* shed from infected animals through other ways including faeces, milk, discharging lesions, saliva, urine and infected genital organs is also possible [5, 6]. When cattle are exposed to *M. bovis*, several factors have a substantial role in subsequent events and the ultimate outcome. Some of these factors are characteristics of the host while others relate to the environment. Examples of risk factors for infection in cattle include age, body condition, breed, climate, farming practices and host genetics [7-9].

The transmission potential of an infectious disease through a population can be measured by the basic reproductive ratio, R_0. It is defined as the expected number of

secondary infections amongst susceptible individuals arising from a single individual animal during its entire infectious period [10]. If R_0 is less than 1 then an infection cannot establish itself as each case gives rise to less than 1 subsequent case; therefore the epidemic will die off. However, when R_0 is greater than 1 then an epidemic can invade [11, 12]. For bTB, this parameter was estimated to be between 1.3 and 1.9 in high risk areas and 0.6 and 1.4 in low risk areas in GB [13]. In another bTB study in the UK, R_0 was estimated to range between 1.02 to 1.11 [14].

1.2 Detection of bovine tuberculosis

When an animal becomes infected with *M. bovis*, initially innate immune defences are triggered, followed by adaptive immune responses which include cell mediated immunity (CMI) [15]. Over time CMI develops and increases, and becomes detectable through the single intra-dermal comparative cervical tuberculin test (SICTT), commonly known as the "skin test". Therefore, bTB diagnosis entails testing for development of CMI to *M. bovis* using tuberculin purified protein derivatives (PPD). The PPD cause a delayed-type hypersensitivity response [15, 16]. The skin test is universally recognised as a diagnostic method and the primary screening test in large-scale field surveillance for bTB. Most countries which implement bTB control and eradication measures, including the United Kingdom (UK) and Republic of Ireland (ROI) use the skin test. During the test, *M. bovis*-PPD is injected intra-dermally into the neck of an animal. To distinguish between animals infected with *M. bovis* and those infected with other *Mycobacterium* species strains, *M. avium*-PPD is also injected adjacent to the *M. bovis*-PPD injection site. Measurements of the difference in size of the reaction to *M. bovis*-PPD compared to *M. avium*-PPD is conducted 72 hours later [17]. The outcome of the skin test determines the disease status of the animal under

two interpretations, the ‘standard’ and the ‘severe’ interpretation. Internationally, the standard interpretation is commonly used, under which an animal can be classified as: 1) “non-reactor” (skin test negative), when the inflammatory response to *M. bovis*-PPD is measured to be less than or equal to that of *M. avium*-PPD; 2) “inconclusive standard reactor”, when response to *M. bovis*-PPD exceeds that of *M. avium*-PPD but not to a degree considered to be clearly indicative of disease and 3) “reactor” (skin test positive), when the reaction is considered to clearly indicate presence of *M. bovis*. In the UK, the minimum size difference between *M. bovis* and *M. avium* PPD reaction that clearly indicates bTB infection is 4 mm [18]. However, in the severe interpretation case, a lower threshold of 2 mm is used to define a reactor [18].

The schedule of events that occur post-testing might differ across countries particularly due to differences in control policies [19]. In the UK and ROI, when a reactor is discovered during routine surveillance testing, the Official Tuberculosis Free (OTF) status of the particular herd is suspended. A new bTB incident, usually referred to as a “breakdown”, is declared and movement of animals from the herd is restricted. In accordance with European Union (EU) testing protocols, further systematic skin tests are conducted in the affected herd at 60-day intervals and all reactors are sent to the abattoir, where macroscopic examination of the carcass for lesions takes place. When lesions are found the reactor is deemed a confirmed case. During the 60-day interval tests, if an inconclusive reactor status remains unresolved after 2 consecutive tests the animal is treated as a reactor and is culled. When a completely negative herd test is obtained, meaning all animals test negatively, the breakdown closes and the herd regains its bTB-free status; however, if confirmed cases have been revealed at the abattoir, two such consecutive negative tests are required for the breakdown to close.

Herds are then re-tested 6 months after the lifting of bTB restrictions and 12 months thereafter, before the herd reverts back to routine surveillance testing [17, 20, 21].

Although widely used, the skin test is not a gold standard diagnostic test due to its imperfection. The contention with skin test as a diagnostic tool lies with its sensitivity and specificity both of which define the accuracy of the test. These characteristics are, respectively, derived from the proportion of infected animals which are correctly diagnosed as positive and the proportion of uninfected animals that are correctly identified as negative [17, 22]. Variable test sensitivity and specificity values have been reported in the literature, with slight differences in how the skin test was implemented. Sensitivity values generally range from 0.51 to 0.81 [17, 23-26] for standard interpretation and 0.61 to 0.93 [17, 24, 25] for severe interpretation. Specificity values for skin test are much higher and estimated between 0.992 and 0.999 [17, 18, 23, 25]. Therefore, possible misdiagnoses are more likely to pertain to false negatives than false positives. However, ease of and wide-spread implementation of the skin test at population level, and the large volume of relevant accumulated data render them useful in large-scale bTB monitoring and eradication programmes in spite of imperfection concerns.

Other bTB diagnostic tests such as the antibody enzyme-linked immuno-assay and the gamma-interferon assay have been proposed as supplementary tests in bTB control and eradication programmes [3]. The gamma-interferon assay has been developed especially to complement the skin test by improving the probability of detecting tuberculous animals thereby enhancing the sensitivity of the diagnosis. This test is used mainly in regions or herds with high incidences of bTB.

As mentioned above, all skin test positive animals are compulsorily slaughtered and undergo post-mortem examination. During post-mortem examination, animals are inspected for visible bTB lesions in their organs. If bTB-like lesions are observed in any of the tissue samples, the animal is classified as a visible lesion (VL) case. Otherwise, if bTB-like lesions are not found during post-mortem examination the animal is classified as a non-visible lesion (NVL) case. Typically, in the UK around 30 to 40% of animals that react positively to the skin test end up as VL cases [27], whereas the remaining 60 to 70% are NVL cases. The NVL phenotype could be indicative of animals in the early stage of infection, a state of latency, low dosage of pathogen and/or other environmental factors, such as exposure of non-infected animals to antigens of environmental mycobacteria that cross-react with *M. bovis* antigens used in the skin test [2, 17]. Tissue samples from a representative number of confirmed VL cases undergo laboratory tests, i.e. histopathology and isolation of *M. bovis* in bacterial culture. Bacteriology as a confirmatory diagnosis has sensitivity and specificity estimated at 0.78 and 0.99, respectively [28]. Confirmation of infection through post-mortem examination (VLs or positive *M. bovis* culture or both) downgrades the herd status from "suspended" to "withdrawn".

Some animals which do not respond to the skin test end up showing signs of infection later. Hence, in addition to skin tests, there is routine abattoir surveillance for bTB through post-mortem meat inspection of non-reactor cattle, whose meat is mainly destined for human consumption. This inspection of animal carcases complements the skin testing programme of cattle on farms. Similarly, whenever an abattoir case is suspected (presence of VLs) the OTF status of the herd of origin is suspended and samples are taken for culture. If infection is confirmed through isolation of *M. bovis*

in the abattoir, the OTF status of the herd is withdrawn. In the UK, the proportion of abattoir cases confirmed by *M. bovis* culture was estimated at 71% in 2013 [27] and 73% in 2014 [29]. However, sensitivity of post mortem inspection at abattoirs is relatively low since it has been found that 47% of animals with lesions are missed during the inspection [4].

1.3 Disease control measures

In countries where bTB was controlled and subsequently eradicated, such as Australia, some EU member states, Canada and some states in the USA, the eradication process was based on regular skin tests, compulsory slaughter of positive reactors, movement restrictions of infected herds and abattoir surveillance [17]. Bio-security measures have also been employed to reduce wildlife-to-cattle and cattle-to-cattle (within and between herds) transmission [30]. Additionally, vaccination strategies have been tested for protection against bTB in cattle [30]. The most common vaccines are based on Bacillus Calmette-Guerin which has been found to significantly reduce the risk of tuberculosis in humans [31]. However, despite substantial investment in the research and development of vaccines, no effective and accessible vaccine is forthcoming. One of the main challenge with the use of vaccines is that they interfere with the diagnostic skin test. The vaccines and other attenuated *M. bovis* strains contain antigens which are present in *M. bovis*-PPD [32], thereby hampering surveillance with the skin test.

Implementation of the conventional approach of testing and compulsory slaughter of infected animals, though instrumental in reducing bTB incidence, has been impeded by the existence of wildlife reservoirs. The Eurasian badger (*Meles meles*) in the UK and ROI [33, 34], the brushtail possum in New Zealand [35, 36] and the white tailed deer in USA [37] have been found to be a maintenance host of *M.*

bovis infection for cattle, consequently influencing the effectiveness of efforts towards eradicating the disease in these countries.

1.4 Worldwide occurrence of bovine tuberculosis

The worldwide annual losses in the agricultural sector due to bTB was estimated at $3-4 billion [38]. The disease has been ranked among the top 10 most important livestock diseases globally [39], mainly due to its importance in developing countries where the disease is endemic, resulting in reduced livestock productivity and animal losses.

Although bTB was once found in many countries worldwide, along the way some of the countries were certified free of the disease as a result of control and eradication programmes. However, bTB continues to be experienced at different prevalence rates in both developed and developing countries. Despite prolonged implementation of control and eradication programmes, bTB prevalence persists in some developed countries in Europe [40], New Zealand [41] and North America [42]. In contrast to developed countries, prevalence data on bTB is generally scarce in developing countries. Nonetheless, information on the disease occurrence and control measures exists and has been reported in Africa, Asia, Latin America and Caribbean countries [3]. A significant number of studies on bTB have been undertaken in African cattle populations [43-47] and wildlife [48-50]. Other countries mainly involved in bTB research are the UK, ROI, Spain, Italy, France, United States, Canada and New Zealand [9].

Due to the potential impact of bTB on international trade of animals and animal products, the disease remains a public health and socio-economic threat worldwide.

The global prevalence of human tuberculosis due to *M. bovis* was estimated at 3.1% of all human tuberculosis cases [51].

Even though bTB remains a public health issue due to its potential for transmission to humans [52], the risk for human infection in the UK and other developed countries has been greatly reduced through pasteurisation of milk, and testing and elimination of infected cattle [9]. However, a different scenario has been observed in developing countries where the disease is widely distributed with no or sporadic application of control measures, and milk pasteurisation being rarely applied [51]. In countries without systematic control policies, the risk of *M. bovis* infection is high, with individuals associated with HIV/AIDS infection being particularly at a higher risk [3].

1.5 Bovine tuberculosis in the United Kingdom

The UK is one of the few European countries that are currently contending with bTB and its effects. Notably, within the UK, Scotland has been certified officially free of bTB (OTF status) since 2009. Meanwhile the governments in England and Wales have set a goal to attain OTF status by 2038 [53], while there are efforts to set such a goal also in Northern Ireland (NI). Historically, the bTB strategy and management in NI differed slightly from Great Britain (GB) owing to independent determination of control policies [19]. According to Abernethy et al. [19], although the disease control measures in GB, NI and ROI remain standardised according to European legislation, differences emanate from different political, geographical and epidemiological features, as well as risk factors for the disease.

In the UK, although voluntary schemes for attested herds were introduced in 1935, it was until 1950 that a national compulsory bTB eradication scheme was

introduced [54]. This involved annual herd testing, compensation for reactors and movement restrictions in affected herds. The scheme was introduced in areas with high bTB prevalence and gradually extended to other areas until the entire UK was covered in 1960. While the number of reactors generally decreased after 1960, the number of bTB incidence in the Southwest of England remained three times higher than in the rest of Great Britain [54]. From then, attention switched to badgers as possible reservoir for cattle infection. Despite badger culling strategies undertaken then, disease incidence rose steadily since 1986 [54]. Notwithstanding this, control and eradication measures of testing and slaughter of reactors, supplemented by routine abattoir surveillance and herd movement restrictions continues but with limited success [55]. Consequently, incidence of bTB in the UK has been marked by a general upward trend in the last decade [56].

The challenge with the major bTB diagnostic tools i.e. skin test and abattoir surveillance resides in their imperfection. As indicated before, sensitivity of the skin test is relatively low hence some infected animals are left behind as false negatives and continue to cause new infections. Similarly, there is possible misdiagnosis at the abattoir due to the low sensitivity of post-mortem inspection [4], hence some possible breakdowns being missed in the process. Increase in bTB incidences have also been exacerbated by presence of badgers which continue to play a role in wildlife-to-cattle infections [34]. It has been indicated that, despite more controls during a breakdown, there has been a tendency for a considerable proportion of breakdowns to recur within 12 months [57]. Consequently, a randomised badger culling trial was undertaken to assess the effectiveness of badger culling in bTB control [58, 59]. However, the outcome indicated that badger culling as a control measure may not be sustainable

especially due to the high cost to benefit ratio of the culling process [60, 61]. Furthermore, this measure instigated considerable political and social controversy.

Due to inadequacies of the current measures to eradicate bTB, the disease has continued to attract high costs towards its control. In 2010/2011, the estimated expenditure by the UK and Irish governments for bTB control was £175 million and £52 million, respectively [19]. In GB, bTB has been reported to be endemic in the southwest and parts of central England and in southwest Wales while occurring sporadically elsewhere [62]. Currently, GB has been sub-divided into a low risk area, a high risk area and an edge area (between low and high risk areas). Surveillance for bTB in GB is carried out through four-yearly herd tests, except in high risk areas where testing is annual [30]. Notably, within the UK the greatest impact due to the number of cattle slaughtered and the financial liability as a result of bTB has been experienced in England and Wales [60].

In England, 29,803 and 30,980 infected cattle were slaughtered in 2016 and 2017, respectively, while the number slaughtered in Wales was 9,444 and 9,693 for the respective years [63]. However, in Scotland only 203 and 150 were slaughtered in 2016 and 2017, respectively [63]. Slaughter of bTB infected cattle results in very high costs, for example in 2009 slaughter of around 25,000 infected cattle costed England £63 million excluding research and development [60].

With continued high expenditure on bTB, it was necessary to consider additional complementary measures to existing bTB control and eradication programmes. One such measure could be to take advantage of host genetic variation in response to the disease [8]. Until recently, the contribution that host genotypes make to the disease outcome has been overlooked by policy makers. According to Driscoll

et al. [56] most of earlier research on bTB in the UK was mainly focused on possible risk factors for exposure such as farm location, herd breakdown history and stocking practices with little attention towards identifying possible genetic factors in the bovine host. Humblet et al. [9] indicated that it was not until recently that the importance of genetics for bTB resistance has started being considered worldwide.

Consequently, exploiting the host genetic variation through breeding for increased bTB resistance within the national herd could produce significant benefits. The benefits would materialise through the use of genetically resistant sires. Such a strategy would have long-term permanent effects thereby complementing the existing control measures in reducing the incidences of the disease [8]. It has been further suggested that identifying genes underlying host susceptibility to bTB infection could further support disease control and facilitate the development of specialised vaccines [21].

1.6 Host genetic variation

Extensive variability in disease occurrence observed among individual animals has been acknowledged to be partially genetic and defined in terms of both within and between breed differences in resistance or susceptibility to various livestock diseases including bTB [64]. Exploration of using selection for genetic improvement should be preceded by confirming the existence and establishing the magnitude of such variation [65]. Several studies involving susceptibility to bTB have been conducted as discussed below.

1.6.1 Overview of within breed genetic variation studies

In selective breeding, one of the most important parameters is trait heritability, which indicates the potential success of the selection strategy for a particular trait. The higher the heritability, the greater the role of genetics on determining the phenotype and the higher the likelihood for enhanced response to selection [66]. Through estimation of heritability, the influence of individual genetic variability on susceptibility to diseases has been investigated for several livestock species. Generally, heritability estimates for disease incidence traits in cattle are low [65, 67, 68]. Heritability estimates across various animal species including cattle have indicated that significant genetic variation for host resistance to bTB exists.

The heritability of resistance to bTB infection in cattle has been estimated in several studies summarised in Table 1.1. So far reported estimates indicate that resistance or susceptibility to bTB is a relatively lowly heritable trait, with conventional pedigree-based heritability estimates ranging between 0.06 and 0.18, and genomic estimates being higher (Table 1.1). Different trait definitions (phenotypes), environmental conditions, populations, breeds and methodologies employed in the various studies contribute to the observed differences. However, in all studies in Table 1.1, some genetic variation was estimated indicating the possibility to selectively breed for enhanced bTB resistance.

Phenotypes used in the computation of heritability estimates for bTB have mainly been based on the disease binary outcome. As shown in Table 1.1 the most commonly explored phenotypes are skin test response (reactors or non-reactors) and confirmed cases (presence or absence of lesions and/or *M. bovis* culture). However, the most recent study of UK dairy cattle (Banos et al. [69]) estimated heritability for

Table 1.1 Summary of heritability estimates for cattle susceptibility to bTB

Species	Breed	Country	bTB phenotype definition	Approach	Heritability	Reference
Dairy cattle	Black-and-white	Russia	Skin test response + confirmed cases	Pedigree	0.06 – 0.08	[70]
Dairy cattle	Holstein-Friesian	ROI	Skin test response	Pedigree	0.14	[71]
Dairy cattle	Holstein-Friesian	ROI	Confirmed cases	Pedigree	0.18	[71]
Dairy cattle	Holstein-Friesian	GB	Skin test response	Pedigree	0.16	[20]
Dairy cattle	Holstein-Friesian	GB	Confirmed cases	Pedigree	0.18	[20]
Dairy cattle	Holstein-Friesian	NI	Confirmed cases	Genomic	0.21	[72]
Dairy cattle	Holstein-Friesian	ROI	Skin test response	Pedigree	0.12	[21]
Beef cattle	Mixed	ROI	Skin test response	Pedigree	0.13	[21]
Dairy cattle	Holstein-Friesian	NI	Confirmed cases	Genomic	0.23	[73]
Dairy cattle	Holstein-Friesian	GB	Skin test response	Pedigree	0.09	[69]
Dairy cattle	Holstein-Friesian	GB	Skin test response + non-reactors and inconclusive reactors with positive post-mortem examination results	Pedigree	0.09	[69]
Dairy cattle	Holstein-Friesian	GB	Confirmed cases	Pedigree	0.12	[69]
Dairy cattle	Holstein-Friesian	NI	Unconfirmed cases (non-visible lesions)	Genomic	0.45	[83]

bTB=bovine tuberculosis; GB=Great Britain; ROI=Republic of Ireland; NI=Northern Ireland; In all cases the traits used to estimate heritability were binary (0, 1) traits from animals involved in bTB breakdowns.

susceptibility to bTB based on non-binary phenotypes including a probability of infection throughout the breakdown.

The correlation between the phenotypes in Table 1.1 has been established. Bermingham et al. [71] reported a high positive genetic correlation (0.99) between response to skin test and confirmed infection. Similarly, Banos et al. [69] found a correlation of 0.62 between estimated breeding values (EBVs) of sires derived from skin test outcomes and confirmed cases. In the same study, a correlation of 0.99 was reported between sire EBVs based on skin test response only and skin test response plus non-reactors and inconclusive reactors with positive post-mortem examination results [69]. A plausible explanation of these results is that disease outcomes from bTB infection are likely controlled by similar genes. Consequently, direct selection for increased resistance based on skin test response will result in reduction of confirmed cases.

In addition to the conventional method of using pedigree information to estimate genetic variation of cattle susceptibility to bTB, other studies used a genomic relationship approach. In the latter, genome-wide data were used and heritability values of 0.21 and 0.23 were estimated in NI Holstein-Friesian cattle [72, 73]. These studies demonstrated the feasibility of genomic selection based on genomic breeding values (predictions). Selection of animals using genomic predictions is advantageous in that estimated breeding values can be obtained without observing phenotypes making it possible to select early in life for bTB resistance, even in populations without pedigree data. Genomic selection, of course, presupposes existence of a well-defined reference population with appropriate phenotypic and genotypic data.

A favourable genetic and phenotypic correlation was reported between milk yield and bTB susceptibility in studies of Brotherstone et al. [20] and Boland et al. [74], respectively. However, Petukhov et al. [70] found no significant differences in milk production between healthy and bTB infected cattle. In the UK, weak and generally favourable genetic correlations were observed between genetic evaluations for bTB on the one hand and genetic evaluations for other traits in the national selection index [69]. In ROI, Bermingham et al. [75] reported that susceptibility to bTB had non-significant genetic correlations with other important dairy traits except fat yield, body condition, milk somatic cell score and longevity.

Apart from cattle, genetic variation in resistance to bTB has also been studied in farmed red deer in New Zealand, where a heritability of 0.48 was estimated based on the skin test outcome in a challenge experiment [76]. This heritability is higher than for most cattle studies. Nonetheless, it has been acknowledged that the heritability for bTB susceptibility from field studies may be underestimated due to the imperfection of the diagnostic test used to measure bTB susceptibility, data recording issues and the unequal exposure to the pathogen among animals in a herd [22].

Besides imperfection of the skin test, another critical question is whether selection against responsiveness to skin test could impair the ability of cattle to mount a detectable response. However, a recent study has indicated that skin test response is lowly heritable (0.01) [77]. That study also estimated heritability of response to skin test in animals <2 years, 2-3 years and >3 years old to be 0.002, 0.019 and 0.015, respectively. Therefore, response to skin test would less likely be affected by selection for resistance in future generations. Furthermore, the continuous skin test outcome in the healthy animals was found to be weakly correlated with skin test positivity (binary

classification of reactor or non-reactor) [77]. Consequently, while genetic selection will reduce the number of cases, it is unlikely to change the specificity of the test.

1.6.2 Overview of across-breed variation studies

From a global perspective, early studies around the 1930s demonstrated that susceptibility levels to bTB differed between breeds. A study in Uganda by Carmichael [78] found that Zebu cattle were more resistant to bTB than taurine Ankole cattle wherein disease incidences of 0.1-0.7% and 12.5-41.4% were reported for the two breeds, respectively. Another study conducted in different cattle breeds (Zebu, Holstein and their crosses) in Ethiopia found the prevalence of bTB in the respective genotypes to be 11.6, 22.2 and 11.9% [79]. Results from that study indicated that the native Zebu breed was more resistant to bTB than the exotic (Holstein) breed. Additionally, the severity of the pathology was found to be more pronounced in the Holstein than the Zebu breed. Similar results showing Zebu cattle being more resistant than other breed types were also reported in a study conducted in Malawi where Zebu, Sussex and Zebu crosses were experimentally challenged with *M. bovis* infection [80]. Between-breed differences in susceptibility to bTB were also reported in ROI [21].

1.6.3 Overview of genomic association studies

Studies addressing the identification of loci associated with susceptibility to bTB began during the last decade. Several methods have been used to address this issue, ranging from candidate gene approach and microsatellite analysis to genome-wide Single Nucleotide Polymorphism (SNP) DNA arrays. In the candidate gene approach, genes potentially involved in biological pathways underlying a trait expression have been employed as molecular markers for quantitative trait loci (QTL) analysis [81].

Polymorphisms of the candidate gene *SLC11A1* have been significantly associated with bTB infection in African Zebu cattle [82] and Holstein dairy cattle in Taiwan [83]. Genetic variants of candidate gene *TLR1* were found to be associated with susceptibility to bTB in Chinese Holstein cattle [84]. Two genomic regions identified by microsatellite markers *INRA111* and *BMS2753* were found to be associated with susceptibility to bTB in UK cattle [56]. All genes involved in these studies play a role in the immunological control of several infectious diseases. Although useful, candidate gene and microsatellite analyses and studies have limitations since in most cases only part of the host genome is involved.

Genome-wide association studies [72, 85, 86] have indicated that susceptibility to bTB may be largely polygenic, meaning it is controlled by many loci. Hence selection based on the entire genome might be the preferred method compared to marker-assisted selection based on specific markers at particular loci [55]. The availability of genome-wide DNA arrays has made it possible to scan the entire genome with the aim to locate regions that are associated with susceptibility to bTB. Most association studies on susceptibility to bTB have been undertaken in the UK and ROI. According to Allen et al. [8] genome-wide association studies (GWAS) could identify the network of genes controlling variation in bTB resistance and shed light on previously undiscovered relevant pathways.

Table 1.2 Summary of genomic study results of susceptibility to bTB in cattle.

Breed	Country	bTB phenotype definition	Dependent variable	Method of analysis	No. records	BTA	SNP base-pair position (bp)	Var_{qtl}	Reference
H/F	ROI	Skin test response	dEBVs	GWAS	307	22	59628616, 59563696, 59588069	$3.5x10^{-6}$	[85]
H/F	NI	Confirmed cases	Case-control	GWAS RHM	1,151	2; 13	25899036; 71782488, 71783216, 71784332, 71787722, 71788784, 71789620, 71791844	*0.022	[72]
Mixed	Ethiopia	Skin test response	Case-control	GWAS	502	6	65162299	-	[87]
H/F	NI/ROI	Skin test response + confirmed cases	dEBVs + case-control (standardised)	RHM CAA	1,438	6	45153840 - 45981562	0.027	[88]
H/F	ROI	Skin test response	dEBVs	GWAS	841	23	9590819-9591806	-	[86]
H/F	NI	Unconfirmed cases (NVLs)	Case-control	RHM CAA	1,359	17; 22; 23	19342437-19650334; 57159725-57547638; 6603103-7066824	0.053; 0.039; 0.035	[89]

bTB = bovine tuberculosis; dEBV = de-regressed estimated breeding value; BTA = *Bos taurus* autosome; SNP = single nucleotide polymorphism; NVLs = skin test positive but non-visible lesions at post-mortem examination

ROI = Republic of Ireland, NI = Northern Ireland; GWAS = Genome-wide association studies; RHM = Regional heritability mapping; CAA = chromosomal association analysis

Var_{qtl} = proportion of phenotypic variance contributed by identified QTL or multiple QTLs*

Genomic association studies using SNP arrays undertaken to identify genomic regions linked with cattle susceptibility to bTB are summarised in Table 1.2. As in the estimation of heritability, phenotypes commonly used in the association studies were based on skin test response and confirmed cases. In most studies phenotype-based dependent variables used were either binary phenotypic records or de-regressed sire EBVs. The latter were produced during the process of estimating sire breeding values and represented aggregate progeny phenotypes adjusted for all environmental and other genetic effects in the model of analysis [90].

The methods used to associate animal genotypes to bTB susceptibility in these studies were GWAS and regional heritability mapping (RHM) analyses. In some studies, chromosomal association was also used to confirm identified regions [88, 89].

GWAS entails regression of a single SNP at a time on the phenotype of interest. This method was used to identify regions associated with bTB susceptibility on *Bos taurus* autosomal chromosomes 2, 6, 13, 22 and 23 [72, 85-87].

Finlay et al. [85] found that the most significant of the three identified SNPs (Table 1.2) on chromosome 22 was found within the taurine transporter gene *SLC6A6* (TauT), whose function related to immune response. The other two SNPs were at high linkage disequilibrium (LD) (r^2=0.9184). GWAS performed in NI dairy cattle identified SNPs on chromosome 13 that were in strong LD with each other. The SNPs were found within the introns of *PTPRT* gene, which has been linked with several non-infectious diseases [72]. Although the GWAS conducted in ROI by Richardson et al. [86] identified several significant SNPs associated with bTB susceptibility on chromosomes 1, 14 and 17, the most significant finding was a region on chromosome

23, which contains *FKBP5* gene that has a role in host immune response to disease in humans.

As an alternative to GWAS, RHM detects genomic regions associated with a trait and assesses the proportion of the total genetic variation emanating from a collective contribution from several individual SNPs located in these regions [91]. In the study of Bermingham et al. [72], RHM identified the same region on chromosome 13 which harbours the seven significant SNPs identified with GWAS. RHM was also applied by Tsairidou et al. [88] in a meta-analysis study on data previously used in ROI [85] and NI [72] which identified a region on chromosome 6 that had not been detected in the previous studies. Kassahun et al. [87] proposed a different genomic region on the same chromosome. Another study based on a trait defined as unconfirmed cases (non-visible lesions) identified regions on chromosomes 17, 22 and 23 associated with susceptibility to bTB using RHM [89]. The respective regional heritabilities were 0.053, 0.039 and 0.035 while the polygenic heritability estimated for this trait was 0.45 [89]. The region identified on chromosome 23 harboured several genes and included members of the *bovine leukocyte antigen class IIB*.

In summary, various studies provided evidence that individual SNPs and/or genomic regions might be associated with bTB resistance/susceptibility in different populations. Interestingly, no common QTLs were identified in these studies. This might be due to different phenotypes, datasets and methods used but may also support the notion of a genetically complex trait in bTB resistance.

1.7 Epidemiological models

While genetic selection for increasing animal resistance to infectious diseases including bTB seems to be possible, it is important to determine the consequences of

selection on disease prevalence and dynamics prior to implementing the breeding programme. As stated by MacKenzie et al. [92], the question is whether selection for resistance will indeed reduce the disease incidence or severity to an acceptable level within a reasonable time period. Understanding the interaction of host genotype and disease epidemiology forms part of the answer to this question. Genetic epidemiological models can incorporate this understanding and quantify the impact of selection on disease epidemiology and transmission dynamics. These models allow for the study of disease epidemiology while accounting for genetic variation in the host. A basic epidemiological model showing the spread of a pathogenic infection through a population was described by Anderson et al. [93]. In the basic compartmental model, animals progress from one stage of the disease to another including the stage where the animal is susceptible but not yet infected and the stage when the animal gets infected with a possibility of later being removed or recover. In a homogeneous population, the inter-compartmental transition rates are equal for all individuals in the same compartment. Host genetic variation implies that transition rates are influenced by the host genetics and thus differ between individuals.

Genetic epidemiological models have been developed for livestock diseases including infectious disease in pigs [94], Mareks in poultry [95], nematode parasitic infection in ruminants [96], footrot in sheep [97] and sea lice in fish [98]. Results from these studies generally indicate that selection for resistance reduces disease prevalence over time. While desirable results can be achieved through selection, in some instances the impact is realised after prolonged periods of selection going as far as 20 generations [97].

Several epidemiological models for bTB have been developed [13, 99-106]. In these models, the various states which an animal can be in during disease progression are: susceptible (*S*); exposed (*E*); test-sensitive (*T*); and infectious (*I*) (Figure 1.1). The transmission coefficient, β, usually impacts on the rate at which cattle-to-cattle transmission of infection occurs. It influences the rate at which animals progress from the *S* (non-infected) to the *E* (infected) state (Figure 1.1). The estimated values of β vary across studies. The within herd transmission rate β estimates reported in literature ranged from 0.006 to 0.014 $days^{-1}$ [13, 99-101, 104]. As is the case, these estimates are expected to vary because the studies were conducted on different cattle populations in different environments and used different estimation methods.

Another key epidemiological parameter is the latency period, i.e. the period between the exposed and the infectious state ($1/\sigma + 1/\gamma$) in Figure 1.1). Unlike human tuberculosis, the latency period of bTB is not well understood. In the *SETI* model (Figure 1.1) used in most bTB modelling studies, there are two sub-stages of the latency period dependent on when an animal becomes responsive to the skin test; the first stage is when animals are infected but not detectable by the skin test, therein called exposed stage (*E*) and the second when they become reactive to the skin test and can be diagnosed as infected, known as the test-sensitive stage (*T*). Animals that react positively to the skin test with sensitivity Ω are removed (*R*) at a rate that depends on the frequency of the test.

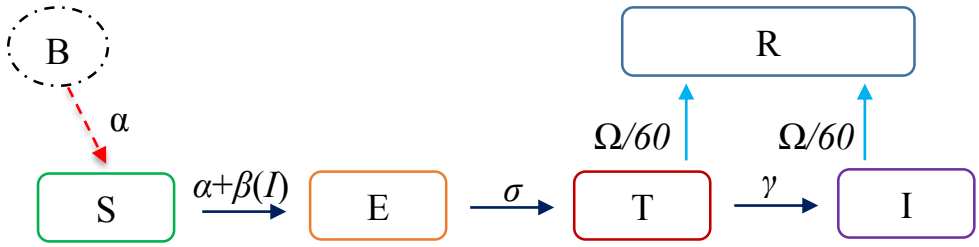

Figure 1.1 Compartmental *SETI* model for a homogeneous population. Different states reflecting critical points in the bTB infection process: susceptible (*S*), exposed (*E*), test-sensitive (*T*) and infectious (*I*). The rate from *S* to *E* state depends on the background infection (α), transmission coefficient (β) and number of infectious (*I*) animals in the herd. Progression from *E* to *T* state and from *T* to *I* state occur at the rate of σ and γ, respectively. Sensitivity of the diagnostic test is represented by Ω while R indicate animals that have been tested and reacted positively to the skin test which are the removed from the herd.

The period from *E* to *T* state reported in bTB models ranged from 22 to 275 days [13, 99, 103, 106]. The reported duration from *T* to *I* state ranged from 28 to 192 days [13, 99, 103, 106]. In an alternative approach, Conlan et al. [99] assumed animals in the *E* (referred to as occult state in their study) and *T* states were infectious, and estimated the time between *E* and *T* to be 1.8 days. O'Hare et al. [13], further studied latency periods separately in high and low bTB risk areas in GB. That study reported durations from *E* to *T* state and from *T* to *I* state of 100 and 190 days, respectively, in high risk areas. Corresponding durations in low risk areas were 60 hours and 180 days.

Other parameters in the classical bTB epidemiological model (Figure 1.1) included the external rate of infection α from wildlife, neighbouring herds and inward moving animals, and the sensitivity of the skin test. In the various studies, the former was estimated between 5×10^{-7} and 0.045 $days^{-1}$ [13, 105, 106], while the latter ranged between 45 and 80% [13, 99, 101-105].

Despite the extensive study of bTB epidemiology, previous bTB models have not accounted for the genetic variation among hosts. Therefore, a genetic epidemiological model for bTB needs to be developed in order to study the impact of genetic selection on the disease dynamics.

1.8 Rationale for current research

The scientific studies summarised above have demonstrated the feasibility of both genetic and genomic selection for increased resistance to bTB. The genetic variation of the hosts (cattle) has not been exploited yet in bTB control and eradication programmes. Genetic and genomic tools may effectively complement current surveillance and culling methods, and decisively contribute to meeting the national UK goal for OTF status by 2038 [30].

Previous genomic association studies have not yet identified a common QTL for resistance to bTB across different populations. Hence more genomic studies are necessary especially on populations that have not been studied before. No genomic studies have yet been conducted on GB cattle.

Disease phenotypes vary across different populations and countries depending on bTB control and management policies, production systems, other environment factors and the genetic background of hosts [19, 20, 107]. The genomic background of novel phenotypes described by Banos et al. [69] has not been established, yet.

As mentioned, before embarking on long-term genetic selection for enhanced bTB resistance, it is worthwhile to quantify the consequences of such a selection process. Firstly, the impact of selection on the prevalence and dynamics of the disease needs to be assessed. Secondly, the impact of genetic selection for bTB resistance on other economically important traits that are currently included in the breeding goal

needs to be understood and quantified. Results from such research would inform and guide the eventual incorporation of bTB in the animal genetic improvement programmes.

1.9 Objectives of the study

The overall aim of this thesis is to address existing scientific research gaps on the genetics of bTB resistance in dairy cattle. The following specific objectives are set for this matter:

1. To identify genomic regions underlying resistance/susceptibility to bTB using novel trait definitions and a new population of study
2. To quantify the impact of long-term genetic selection for increased resistance to bTB on disease prevalence and dynamics
3. To determine the consequences of genetically selecting animals for increased resistance to bTB on other economically important dairy cattle traits

This PhD thesis is written in five chapters comprising the General introduction (Chapter 1), three technical Chapters addressing each of the above objectives and a Discussion Chapter. Chapter 2 of the thesis addresses the first objective and describes genomic studies of three trait definitions on bTB infection in the GB Holstein-Frisian population. Chapter 3 describes and evaluates the impact of long-term selection for increased bTB resistance on the disease dynamics using a genetic epidemiological model thereby addressing the second objective. Chapter 4 addresses the third objective by investigating consequences of selection for bTB resistance on other dairy traits and Chapter 5 provides an overall discussion, which includes suggestions for practical application of study results and possible future research. A different set of published references is cited and listed in each Chapter.

1.10 References

1. Green LE, Cornell SJ. Investigations of cattle herd breakdowns with bovine tuberculosis in four counties of England and Wales using VETNET data. Prev Vet Med. 2005; 70(3–4):293-311.
2. Corner L. Post mortem diagnosis of Mycobacterium bovis infection in cattle. Vet Microbiol. 1994; 40(1-2):53-63.
3. Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I. Bovine tuberculosis: an old disease but a new threat to Africa. Int J Tuberc Lung Dis. 2004; 8(8):924-937.
4. Corner L, Melville L, McCubbin K, Small K, McCormick B, Wood P, Rothel J. Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. Aust Vet J. 1990; 67(11):389-392.
5. Neill S, Pollock J, Bryson D, Hanna J. Pathogenesis of Mycobacterium bovis infection in cattle. Vet Microbiol. 1994; 40(1-2):41-52.
6. Phillips C, Foster C, Morris P, Teverson R. The transmission of Mycobacterium bovis infection to cattle. Res Vet Sci. 2003; 74(1):1-15.
7. Pollock JM, Neill SD. Mycobacterium bovis infection and tuberculosis in cattle. Vet J. 2002; 163(2):115-127.
8. Allen AR, Minozzi G, Glass EJ, Skuce RA, McDowell SWJ, Woolliams JA, Bishop SC. Bovine tuberculosis: the genetic basis of host susceptibility. Proc R Soc Lond B Biol Sci. 2010; 277(1695):2737-2745.
9. Humblet M-F, Boschiroli ML, Saegerman C. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet Res. 2009; 40(5):1-24.

10. Heffernan J, Smith R, Wahl L. Perspectives on the basic reproductive ratio. J R Soc Interface. 2005; 2(4):281-293.

11. Roberts M, Heesterbeek H. Bluff your way in epidemic models. Trends Microbiol. 1993; 1(9):343-348.

12. Diekmann O, Heesterbeek JAP, Metz JA. On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases in heterogeneous populations. J Math Biol. 1990; 28(4):365-382.

13. O'Hare A, Orton RJ, Bessell PR, Kao RR. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. Proc R Soc Lond B Biol Sci. 2014; 281(1783).

14. Cox D, Donnelly CA, Bourne FJ, Gettinby G, McInerney JP, Morrison WI, Woodroffe R. Simple model for tuberculosis in cattle and badgers. Proc Natl Acad Sci USA. 2005; 102(49):17588-17593.

15. Pollock J, McNair J, Welsh M, Girvin R, Kennedy H, Mackie D, Neill S. Immune responses in bovine tuberculosis. Tuberculosis. 2001; 81(1-2):103-107.

16. Welsh MD, Cunningham RT, Corbett DM, Girvin RM, McNair J, Skuce RA, Bryson DG, Pollock JM. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology. 2005; 114(1):101-111.

17. De la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci. 2006; 81(2):190-210.

18. Goodchild A, Downs S, Upton P, Wood J, De la Rua-Domenech R. Specificity of the comparative skin test for bovine tuberculosis in Great Britain. Vet Rec. 2015; 177(10):258-258.

19. Abernethy DA, Upton P, Higgins IM, McGrath G, Goodchild AV, Rolfe SJ, et al. Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995-2010. Vet Rec. 2013; 172(12):312.

20. Brotherstone S, White I, Coffey M, Downs S, Mitchell A, Clifton-Hadley R, More S, Good M, Woolliams J. Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*. J Dairy Sci. 2010; 93(3):1234-1242.

21. Richardson IW, Bradley DG, Higgins IM, More SJ, McClure J, Berry DP. Variance components for susceptibility to *Mycobacterium bovis* infection in dairy and beef cattle. Genet Sel Evol. 2014; 46(1):1.

22. Bishop SC, Woolliams JA. On the genetic interpretation of disease data. PLoS One. 2010; 5(1):e8940.

23. Clegg TA, Duignan A, Whelan C, Gormley E, Good M, Clarke J, Toft N, More SJ. Using latent class analysis to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Vet Microbiol. 2011; 151(1):68-76.

24. Karolemeas K, de la Rua-Domenech R, Cooper R, Goodchild AV, Clifton-Hadley RS, Conlan AJ, et al. Estimation of the relative sensitivity of the comparative tuberculin skin test in tuberculous cattle herds subjected to depopulation. PLoS One. 2012; 7(8):e43217.

25. Álvarez J, Perez A, Bezos J, Marqués S, Grau A, Saez JL, Mínguez O, de Juan L, Domínguez L. Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Vet Microbiol. 2012; 155(1):38-43.

26. Downs S, Parry J, Nunez-Garcia J, Abernethy D, Broughan J, Cameron A, et al. Meta-analysis of diagnostic test performance and modelling of testing strategies for control of bovine tuberculosis in GB. Proc of Society for Veterinary Epidemiology and Preventive Medicine. 2011:139-153.

27. Broughan J, Harris K, Brouwer A, Downs S, Goodchild A, Upton P, Smith N. Bovine TB infection status in cattle in Great Britain in 2013. Vet Rec. 2015; 176(13):326-330.

28. Courcoul A, Moyen J-L, Brugere L, Faye S, Henault S, Gares H, Boschiroli M-L. Estimation of sensitivity and specificity of bacteriology, histopathology and PCR for the confirmatory diagnosis of bovine tuberculosis using latent class analysis. PloS one. 2014; 9(3):e90334.

29. Lawes J, Harris K, Brouwer A, Broughan J, Smith N, Upton P. Bovine TB surveillance in Great Britain in 2014. Vet Rec. 2016; 178(13):310-315.

30. Department for Environment Food and Rural Affairs (DEFRA). The strategy for achieving officially bovine tuberculosis free status for England. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/300447/pb14088-bovine-tb-strategy-140328.pdf (Accessed 25th May 2017).

31. Brewer TF. Preventing tuberculosis with bacillus Calmette-Guerin vaccine: a meta-analysis of the literature. Clin Infect Dis. 2000; 31(Supplement 3):S64-S67.

32. Buddle BM, Parlane NA, Keen DL, Aldwell FE, Pollock JM, Lightbody K, Andersen P. Differentiation between Mycobacterium bovis BCG-vaccinated and M. bovis-infected cattle by using recombinant mycobacterial antigens. Clin Diagn Lab Immunol. 1999; 6(1):1-5.

33. Gormley E, Collins J. The development of wildlife control strategies for eradication of tuberculosis in cattle in Ireland. Tubercle Lung Dis. 2000; 80(4-5):229-236.

34. Gallagher J, Clifton-Hadley R. Tuberculosis in badgers; a review of the disease and its significance for other animals. Res Vet Sci. 2000; 69(3):203-217.

35. Coleman J, Cooke M. Mycobacterium bovis infection in wildlife in New Zealand. Tuberculosis. 2001; 81(3):191-202.

36. Coleman J, Coleman M, Warburton B. Trends in the incidence of tuberculosis in possums and livestock, associated with differing control intensities applied to possum populations. New Zeal Vet J. 2006; 54(2):52-60.

37. Payeur JB, Church S, Mosher L, Robinson-Dunn B, Schmitt S, Whipple D. Bovine tuberculosis in Michigan wildlife. Ann N Y Acad Sci. 2002; 969(1):259-261.

38. Thoen C, Steele J. Mycobacterium bovis infection in animals and humans: Iowa State University Press, Ames, USA; 1995.

39. Perry B, Randolph T, McDermott J, Sones K, Thornton P. Investing in animal health research to alleviate poverty. Nairobi, Kenya: ILRI (International Livestock Research Institute); 2002.

40. Schiller I, Waters WR, Vordermeier HM, Jemmi T, Welsh M, Keck N, et al. Bovine tuberculosis in Europe from the perspective of an officially

tuberculosis free country: Trade, surveillance and diagnostics (vol 151, pg 152, 2011). Vet Microbiol. 2012; 155(2-4):448-448.

41. Tweddle NE, Livingstone P. Bovine tuberculosis-control and eradication programs in Australia and New-Zealand. Vet Microbiol. 1994; 40(1-2):23-39.

42. Essey MA, Koller MA. Status of bovine tuberculosis in North-America. Vet Microbiol. 1994; 40(1-2):15-22.

43. Kazwala R, Kambarage D, Daborn C, Nyange J, Jiwa S, Sharp J. Risk factors associated with the occurrence of bovine tuberculosis in cattle in the Southern Highlands of Tanzania. Vet Res Commun. 2001; 25(8):609-614.

44. Munyeme M, Muma J, Samui K, Skjerve E, Nambota A, Phiri I, Rigouts L, Tryland M. Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. Trop Anim Health Prod. 2009; 41(3):345.

45. Tschopp R, Schelling E, Hattendorf J, Aseffa A, Zinsstag J. Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. Prev Vet Med. 2009; 89(3):205-211.

46. Müller B, Hilty M, Berg S, Garcia-Pelayo MC, Dale J, Boschiroli ML, et al. African 1, an epidemiologically important clonal complex of Mycobacterium bovis dominant in Mali, Nigeria, Cameroon, and Chad. J Bacteriol. 2009; 191(6):1951-1960.

47. Awah Ndukum J, Kudi AC, Bradley G, Ane-Anyangwe I, Fon-Tebug S, Tchoumboue J. Prevalence of bovine tuberculosis in abattoirs of the littoral and Western highland regions of cameroon: a cause for public health concern. Vet Med Int. 2010; 2010:Article ID 495015.

48. Michel A, Bengis RG, Keet D, Hofmeyr M, De Klerk L, Cross P, et al. Wildlife tuberculosis in South African conservation areas: implications and challenges. Vet Microbiol. 2006; 112(2):91-100.

49. Cleaveland S, Mlengeya T, Kazwala R, Michel A, Kaare M, Jones S, Eblate E, Shirima G, Packer C. Tuberculosis in Tanzanian wildlife. J Wildl Dis. 2005; 41(2):446-453.

50. Michel AL. Implications of tuberculosis in African wildlife and livestock. Ann N Y Acad Sci. 2002; 969(1):251-255.

51. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, et al. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerging Infect Dis. 1998; 4(1):59-70.

52. Meade KG, Gormley E, Doyle MB, Fitzsimons T, O'Farrelly C, Costello E, Keane J, Zhao Y, MacHugh DE. Innate gene repression associated with Mycobacterium bovis infection in cattle: toward a gene signature of disease. BMC Genomics. 2007; 8(1):400.

53. Department for Environment Food and Rural Affairs (DEFRA). Quarterly publication of national statistics on the incidence and prevalence of tuberculosis (TB) in cattle in Great Britain – to end December 2016. https://www.gov.uk/government/uploads/system/uploads/attachmentdata/file/599784/bovinetb-statsnotice-quarterly-15Mar17.pdf (Accessed 25th May 2017).

54. De la Rua-Domenech R. Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis. 2006; 86(2):77-109.

55. Bishop SC, Woolliams JA. Genomics and disease resistance studies in livestock. Livestock Science. 2014; 166:190-198.

56. Driscoll EE, Hoffman JI, Green LE, Medley GF, Amos W. A preliminary study of genetic factors that influence susceptibility to bovine tuberculosis in the British cattle herd. PLoS One. 2011; 6(4):e18806.

57. Karolemeas K, McKinley TJ, Clifton-Hadley RS, Goodchild AV, Mitchell A, Johnston WT, Conlan AJK, Donnelly CA, Wood JLN. Recurrence of bovine tuberculosis breakdowns in Great Britain: risk factors and prediction. Prev Vet Med. 2011; 102(1):22-29.

58. Donnelly CA, Wei G, Johnston WT, Cox D, Woodroffe R, Bourne FJ, et al. Impacts of widespread badger culling on cattle tuberculosis: concluding analyses from a large-scale field trial. Int J Infect Dis. 2007; 11(4):300-308.

59. Donnelly CA, Woodroffe R, Cox D, Bourne J, Gettinby G, Le Fevre AM, McInerney JP, Morrison WI. Impact of localized badger culling on tuberculosis incidence in British cattle. Nature. 2003; 426(6968):834-837.

60. Johnston WT, Vial F, Gettinby G, Bourne FJ, Clifton-Hadley RS, Cox DR, et al. Herd-level risk factors of bovine tuberculosis in England and Wales after the 2001 foot-and-mouth disease epidemic. Int J Infect Dis. 2011; 15(12):E833-E840.

61. Brunton LA, Donnelly CA, O'Connor H, Prosser A, Ashfield S, Ashton A, et al. Assessing the effects of the first 2 years of industry-led badger culling in England on the incidence of bovine tuberculosis in cattle in 2013–2015. Ecology and evolution. 2017; 7(18):7213-7230.

62. Gilbert M, Mitchell A, Bourn D, Mawdsley J, Cliton-Hadley R, Wint W. Cattle movements and bovine tuberculosis in Great Britain. Nature. 2005; 435(7041):491-496.

63. Department for Environment Food and Rural Affairs (DEFRA). Quarterly publication of national statistics on the incidence and prevalence of tuberculosis (TB) in cattle in Great Britain – to end June 2017. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/645758/bovinetb-statsnotice-quarterly-20sep17.pdf (Accessed 24th November 2017).

64. Morris CA. A review of genetic resistance to disease in Bos taurus cattle. Vet J. 2007; 174(3):481-491.

65. Koets AP, Adugna G, Janss LLG, van Weering HJ, Kalis CHJ, Wentink GH, Rutten V, Schukken YH. Genetic variation of susceptibility to Mycobacterium avium subsp paratuberculosis infection in dairy cattle. J Dairy Sci. 2000; 83(11):2702-2708.

66. Falconer DS. Introduction to quantitative genetics, 4th edn. Harlow, UK: Pearson Education Limited; 1996.

67. Mrode R, Swanson G. Genetic and statistical properties of somatic cell count and its suitability as an indirect means of reducing the incidence of mastitis in dairy cattle. Anim Breed Abstr. 1996; 64:847-857.

68. Heringstad B, Chang YM, Gianola D, Klemetsdal G. Genetic analysis of clinical mastitis, milk fever, ketosis, and retained placenta in three lactations of Norwegian Red cows. J Dairy Sci. 2005; 88(9):3273-3281.

69. Banos G, Winters M, Mrode R, Mitchell A, Bishop SC, Woolliams JA, Coffey MP. Genetic evaluation for bovine tuberculosis resistance in dairy cattle. J Dairy Sci. 2016; 100(2):1272-1281.

70. Petukhov V, Kochnev N, Panov B, Korotkevich O, Kulikova S, Marenkov V. Genetics of cattle resistance to tuberculosis. In: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Amidale, Australia, 11-16 January. 1998.

71. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Brotherstone S, Berry DP. Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. J Dairy Sci. 2009; 92(7):3447-3456.

72. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, et al. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity. 2014; 112(5):543-551.

73. Tsairidou S, Woolliams JA, Allen AR, Skuce RA, McBride SH, Wright DM, et al. Genomic prediction for tuberculosis resistance in dairy cattle. PLoS One. 2014; 9(5):e96728.

74. Boland F, Kelly G, Good M, More S. Bovine tuberculosis and milk production in infected dairy herds in Ireland. Prev Vet Med. 2010; 93(2):153-161.

75. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Berry DP. Genetic correlations between measures of Mycobacterium bovis infection and economically important traits in Irish Holstein-Friesian dairy cows. J Dairy Sci. 2010; 93(11):5413-5422.

76. Mackintosh CG, Qureshi T, Waldrup K, Labes RE, Dodds KG, Griffin JFT. Genetic resistance to experimental infection with Mycobacterium bovis in red deer (Cervus elaphus). Infect Immun. 2000; 68(3):1620-1625.

77. Tsairidou S, Brotherstone S, Coffey M, Bishop SC, Woolliams JA. Quantitative genetic analysis of the bTB diagnostic single intradermal comparative cervical test (SICCT). Genet Sel Evol. 2016; 48(1):90.

78. Carmichael J. Bovine tuberculosis in the tropics, with special reference to Uganda: Part I. J Comp Pathol Therap. 1939; 52:322-335.

79. Ameni G, Aseffa A, Engers H, Young D, Gordon S, Hewinson G, Vordermeier M. High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. Clin Vaccine Immunol. 2007; 14(10):1356-1361.

80. Ellwood DC, Waddington FG. Second experiment to challenge resistance to tuberculosis in bcg vaccinated cattle in Malawi. Br Vet J. 1972; 128(12):619-626.

81. Faris J, Li W, Liu D, Chen P, Gill B. Candidate gene analysis of quantitative disease resistance in wheat. Theor Appl Genet. 1999; 98(2):219-225.

82. Kadarmideen H, Ali A, Thomson P, Müller B, Zinsstag J. Polymorphisms of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. Anim Genet. 2011; 42(6):656-658.

83. YaFen C, ChensHen H, HsiangJung T. Relationship of bovine SLC11A1 (formerly NRAMP1) polymorphisms to the risk of bovine tuberculosis in Holstein cattle. J Vet Sci Techno. 2015; 6(5).

84. Sun L, Song Y, Riaz H, Yang H, Hua G, Guo A, Yang L. Polymorphisms in toll-like receptor 1 and 9 genes and their association with tuberculosis susceptibility in Chinese Holstein cattle. Vet Immunol Immunopathol. 2012; 147(3):195-201.

85. Finlay EK, Berry DP, Wickham B, Gormley EP, Bradley DG. A genome wide association scan of bovine tuberculosis susceptibility in Holstein-Friesian dairy cattle. PLoS One. 2012; 7(2):e30545.

86. Richardson IW, Berry DP, Wiencko HL, Higgins IM, More SJ, McClure J, Lynn DJ, Bradley DG. A genome-wide association study for genetic susceptibility to *Mycobacterium bovis* infection in dairy cattle identifies a susceptibility QTL on chromosome 23. Genet Sel Evol. 2016; 48(1):1.

87. Kassahun Y, Mattiangeli V, Ameni G, Hailu E, Aseffa A, Young DB, Hewinson RG, Vordermeier HM, Bradley DG. Admixture mapping of tuberculosis and pigmentation-related traits in an African-European hybrid cattle population. Front Genet. 2015; 6:210.

88. Tsairidou S, Woolliams J, Allen A, Skuce R, McBride S, Pong-Wong R, et al. A meta-analysis for bovine tuberculosis resistance in dairy cattle. In. Proceedings of the British Society of Animal Science, Chester, UK, 14-15 April; 2015.

89. Wilkinson S, Bishop S, Allen A, McBride S, Skuce R, Bermingham M, Woolliams J, Glass E. Fine-mapping host genetic variation underlying outcomes to Mycobacterium bovis infection in dairy cows. BMC Genomics. 2017; 18(1):477.

90. Garrick DJ, Taylor JF, Fernando RL. Deregressing estimated breeding values and weighting information for genomic regression analyses. Genet Sel Evol. 2009; 41(1):1.

91. Nagamine Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Rudan I, et al. Localising loci underlying complex trait variation using regional genomic relationship mapping. PLoS One. 2012; 7(10):e46501.

92. MacKenzie K, Bishop SC. Utilizing stochastic genetic epidemiological models to quantify the impact of selection for resistance to infectious diseases in domestic livestock. J Anim Sci. 2001; 79(8):2057-2065.

93. Anderson RM, May RM, Anderson B. Infectious diseases of humans: dynamics and control: Oxford University Press, Oxford, UK; 1992.

94. MacKenzie K, Bishop SC. Developing stochastic epidemiological models to quantify the dynamics of infectious diseases in domestic livestock. J Anim Sci. 2001; 79(8):2047-2056.

95. Nath M. Development of a genetic epidemiological model for Marek's disease in poultry. In: Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, 13-18 August. 2006.

96. Bishop SC, Stear MJ. Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. Vet Parasitol. 2003; 115(2):147-166.

97. Nieuwhof GJ, Conington J, Bishop SC. A genetic epidemiological model to describe resistance to an endemic bacterial disease in livestock: application to footrot in sheep. Genet Sel Evol. 2009; 41(1):19.

98. Gharbi K, Matthews L, Bron J, Roberts R, Tinch A, Stear M. The control of sea lice in Atlantic salmon by selective breeding. J R Soc Interface. 2015; 12(110):20150574.

99. Conlan AJ, McKinley TJ, Karolemeas K, Pollock EB, Goodchild AV, Mitchell AP, Birch CP, Clifton-Hadley RS, Wood JL. Estimating the hidden burden of bovine tuberculosis in Great Britain. PLoS Comp Biol. 2012; 8(10):e1002730.

100. Perez AM, Ward MP, Charmandarián A, Ritacco V. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. Prev Vet Med. 2002; 54(4):361-372.

101. Bekara MEA, Courcoul A, Benet J-J, Durand B. Modeling tuberculosis dynamics, detection and control in cattle herds. PLoS One. 2014; 9(9):e108584.

102. Kao R, Roberts M, Ryan T. A model of bovine tuberculosis control in domesticated cattle herds. Proc R Soc Lond B Biol Sci. 1997; 264(1384):1069-1076.

103. Barlow N, Kean J, Hickling G, Livingstone P, Robson A. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Prev Vet Med. 1997; 32(1-2):57-75.

104. Fischer E, Van Roermund H, Hemerik L, Van Asseldonk M, De Jong M. Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev Vet Med. 2005; 67(4):283-301.

105. Brooks-Pollock E, Roberts GO, Keeling MJ. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature. 2014; 511(7508):228-231.

106. Biek R, O'Hare A, Wright D, Mallon T, McCormick C, Orton RJ, et al. Whole genome sequencing reveals local transmission patterns of Mycobacterium bovis in sympatric cattle and badger populations. PLoS Pathog. 2012; 8(11):e1003008.

107. Riggio V, Pong-Wong R, Sallé G, Usai MG, Casu S, Moreno C, Matika O, Bishop SC. A joint analysis to identify loci underlying variation in nematode resistance in three European sheep populations. J Anim Breed Genet. 2014; 131(6):426-436.

CHAPTER 2

Genomic regions underlying susceptibility to bovine tuberculosis in Holstein-Friesian cattle

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2.1 Introduction

This chapter addresses the first objective of the thesis and comprises a scientific manuscript that was published in BMC Genetics (https://doi.org/10.1186/s12863-017-0493-7). Genome-wide association studies, regional heritability mapping and chromosomal association analyses were applied to identify genomic regions that are associated with bovine tuberculosis (bTB). Genomic regions were identified by regressing three bTB phenotypes on animal genotypes based on 50K SNP DNA arrays. Phenotypes were de-regressed estimated breeding values of Holstein-Friesian dairy sires derived from the official national genetic evaluation for bTB in the UK. Chapter 2 also quantifies the amount of genetic variation in resistance/susceptibility to bTB that exists within the studied population.

The student performed the analyses and wrote the manuscript under guidance from the supervisors and in collaboration with co-authors.

2.2 Genomic regions underlying susceptibility to bovine tuberculosis in Holstein-Friesian cattle

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2.2.1 Abstract

Background: The significant social and economic loss as a result of bovine tuberculosis (bTB) presents a continuous challenge to cattle industries in the UK and worldwide. However, host genetic variation in cattle susceptibility to bTB provides an opportunity to select for resistant animals and further understand the genetic mechanisms underlying disease dynamics.

Results: The present study identified genomic regions associated with susceptibility to bTB using genome-wide association (GWA), regional heritability mapping (RHM) and chromosome association approaches. Phenotypes comprised de-regressed estimated breeding values of 804 Holstein-Friesian sires and pertained to three bTB indicator traits: i) positive reactors to the skin test with positive post-mortem examination results (phenotype 1); ii) positive reactors to the skin test regardless of post-mortem examination results (phenotype 2) and iii) as in (ii) plus non-reactors and inconclusive reactors to the skin tests with positive post-mortem examination results (phenotype 3). Genotypes based on the 50K SNP DNA array were available and a total

of 34,874 SNPs remained per animal after quality control. The estimated polygenic heritability for susceptibility to bTB was 0.26, 0.37 and 0.34 for phenotypes 1, 2 and 3, respectively. GWA analysis identified a putative SNP on *Bos taurus* autosomes (BTA) 2 associated with phenotype 1, and another on BTA 23 associated with phenotype 2. Genomic regions encompassing these SNPs were found to harbour potentially relevant annotated genes. RHM confirmed the effect of these genomic regions and identified new regions on BTA 18 for phenotype 1 and BTA 3 for phenotypes 2 and 3. Heritabilities of the genomic regions ranged between 0.05 and 0.08 across the three phenotypes. Chromosome association analysis indicated a major role of BTA 23 on susceptibility to bTB.

Conclusion: Genomic regions and candidate genes identified in the present study provide an opportunity to further understand pathways critical to cattle susceptibility to bTB and enhance genetic improvement programmes aiming at controlling and eradicating the disease.

Keywords: Bovine tuberculosis, Susceptibility, Genome-wide association, Regional heritability mapping, Chromosome association

2.2.2 Background

Bovine tuberculosis (bTB) is a chronic disease caused by *Mycobacterium bovis (M. bovis)* and usually manifests with tuberculous lesions predominantly in the respiratory tract, although lesions could also be found elsewhere [1]. Despite the implementation of nationwide compulsory bTB eradication schemes that were introduced in the United Kingdom in 1950 [2], the incidence of bTB has been marked by a general upward trend since the 1990s [3] resulting in large financial losses for the bovine industry. In Great Britain, the greatest impact of animal and financial losses are experienced in South-

Western England and Wales [4]. During 2010/2011, an estimated £152 million was spent on management and control of the disease in these areas [5]. Scotland was certified officially free of bTB (OTF) in 2009 [6].

In Great Britain, bTB control and eradication programme involves routine testing and compulsory slaughter of infected animals and cattle movement restrictions in the affected herds. Routine testing is based on the administration of the single intradermal comparative cervical tuberculin (SICCT) or 'skin' test to each animal, which entails simultaneous injection of both *M. bovis* and *M. avium* tuberculins side-by-side into the skin of the neck, followed by examination for evidence of localised inflammation after 72 hours. Interpretation of the test follows a standard procedure applied internationally [7]. When reaction to *M. bovis* tuberculin injection is estimated to be less than or equal to that to *M. avium* tuberculin injection then the skin test is deemed negative. A positive skin test result, also known as a 'reactor', is asserted when the reaction to *M. bovis* tuberculin exceeds that to *M. avium* tuberculin by more than 4 mm. In all other cases, the test is considered inconclusive and retesting is done at 60-day intervals to resolve their status. A breakdown (bTB incident) is declared once at least one reactor is discovered in a herd, prompting animal movement restrictions, suspension of the OTF status of the herd and testing of all animals in the herd at 60-day interval. Animals with a positive or two consecutive inconclusive skin tests are slaughtered and examined at the abattoir for visible lesions of bTB in their organs. Samples of tissue from a representative number of infected animals from each breakdown are sent to the laboratory where *M. bovis* culture is performed. A positive post-mortem examination result, i.e. presence of lesions and/or positive *M. bovis* culture (confirmed case) elicits a change of the herd's OTF status from 'suspended' to

'withdrawn'. The breakdown remains 'open' and skin testing continues in the herd until two consecutive negative herd tests are obtained.

Given the difficulties in eradicating bTB, breeding for resistance has been considered as an additional complementary control measure [8]. Most of earlier research on bTB was mainly focused on environmental risk factors for bTB infection [9-11], whilst limited attention was given towards identifying possible genetic factors in the bovine host. However, it was not until recently that genetic studies established the presence of between animal variation in dairy and beef cattle susceptibility to the disease with heritability estimates ranging between 0.09 and 0.23 [12-16]. Furthermore, some genome-wide association (GWA) and regional heritability mapping (RHM) analyses aiming at identifying Quantitative Trait Loci (QTL) underlying cattle susceptibility to bTB have been undertaken. GWA analysis by Finlay et al. [17] and Richardson et al. [18] identified genomic regions associated with bTB susceptibility on *Bos taurus* autosomes (BTA) 22 and 23, respectively, in Irish Holstein-Friesian dairy cattle. Bermingham et al. [19] found regions on BTA 13 in Northern Irish Holstein-Friesian dairy cattle using both GWA and RHM approaches. Tsairidou et al. [20] applied RHM to perform a meta-analysis using the datasets from previous studies in the Republic of Ireland [17] and Northern Ireland [19], and identified a new region on BTA 6. Furthermore, Kassahun et al. [21] also identified a SNP on BTA 6 associated with bTB in a mixed breed cattle population in Ethiopia; however, this region was distinct from that of Tsairidou et al. [20]. In general, genomic studies performed to date have not revealed any major common QTL; therefore further studies with independent populations are required.

Our objective was to conduct a first study of the genomic architecture of susceptibility to bTB in the British Holstein-Friesian cattle population. We used GWA, RHM and chromosome association approaches to analyse alternative definitions of bTB susceptibility that have not been genomically addressed before.

2.2.3 Methods

2.2.3.1 Phenotypes

Data for the present study were sire genetic evaluations that had been previously generated from the official genetic and genomic evaluation system for bTB resistance [15, 22]. These genetic evaluations had been based on skin test and post-mortem examination records of Holstein-Friesian cows obtained from breakdowns (herds with bTB incidents) that occurred between the years 2000 and 2014. Susceptibility to bTB was based on the health status of each animal in a breakdown, i.e. either infected (case) or healthy (control). Three alternative definitions of "infected" from Banos et al. [15] were considered:

i) Phenotype 1: positive reactors to the skin test with positive post-mortem examination results consisting of visible lesions of bTB and/or positive *M. bovis* culture. This phenotype represented the conservative definition of infected, which requires infection to be confirmed by post-mortem examination.

ii) Phenotype 2: positive reactors to the skin test regardless of post-mortem examination results, based on the very high specificity of the skin test (ca. 99%) and the trivial number of false positives expected [7] . Phenotype 2 included all phenotype 1 animals and those without positive post-mortem examination results.

iii) Phenotype 3: as in (ii) plus non-reactors and inconclusive reactors to the skin test who had been slaughtered and had positive post-mortem examination results, in order to include possible false negative skin tests in this definition [8]. The majority (97.3%) of this phenotype included phenotype 2 animals plus a few inconclusive (2.6%) and non-reactors (0.1%) to the skin test.

In all cases, healthy animals were defined as live non-reactors to the skin test or slaughtered non-reactors with negative post-mortem examination results. Animals defined as healthy were all from the same breakdowns as the infected ones.

Following the above trait definitions, a linear mixed model was used to calculate sire EBVs based on the phenotypes of their daughters. Each sire received three EBVs, one for each of the above trait definitions. More information about the genetic model used to derive these sire EBVs may be found in Banos et al. [15]. In the current study, sire EBVs were de-regressed and used as phenotypes. The de-regression was necessary because actual EBVs have been found to be unsuitable phenotypes for GWAS as they are usually regressed depending on pedigree structure and number of daughters per sire, and also include familial information all of which have the potential to reduce power, increase the rate of false positive results and misestimate QTL effect size [23]. The de-regression process accounted for sire EBV reliability and parental average effects, and followed the procedure described by Garrick et al. [24]. Consistent with the common genetic evaluation practice, de-regression was applied to sire EBVs with a minimum reliability of 0.30.

2.2.3.2 Genotypes

Whole-genome genotypes based on the 50K SNP Illumina BeadChip were available for 804 Holstein-Friesian sires with de-regressed EBVs for susceptibility to bTB. Genotype data were subjected to quality control using the software PLINK [25]. Quality control removed SNPs with minor allele frequency below 0.05 and call rates below 0.90, and significantly deviated from Hardy-Weinberg equilibrium ($P < 1x10^{-6}$). Quality control also removed animals with individual call rates below 0.90. A total of 34,874 autosomal SNPs and 803 individuals passed the quality control criteria and were retained for the subsequent analyses.

The genomic data (sire genotypes) were explored for underlying population substructure using multi-dimensional scaling based on the genomic kinship matrix estimated from all SNPs in the analysis. The genomic kinship matrix was calculated as outlined by Amin et al. [26].

Subsequently, three alternative approaches were used to test for associations of genotypes with bTB susceptibility traits: GWA, RHM and chromosome association analyses. Each bTB trait was analysed separately. Prior to the association analyses, de-regressed EBVs were weighted using the formula outlined by Garrick et al. [24]:

$$\omega_i = \frac{1-h^2}{[c+(1-r_i^2)/r_i^2]h^2}$$

where ω_i is the weighting factor of the de-regressed EBV of the *i*th animal; h^2 is the heritability of the trait ($h^2 = 0.09$ [15]); r_i^2 is the reliability of the de-regressed EBV of the *i*th sire and c is the genetic variance not accounted for by the SNPs. A value of 0.20 [27] was considered for c.

Furthermore, Pearson correlations between the three sets of sire EBVs were calculated.

2.2.3.3 Genome-wide association analysis

GWA analysis was performed by regressing the de-regressed EBV on each individual SNP using the following model:

$$y = \mu + Xb + Za + e \qquad (1)$$

where y is a vector of observations on the trait (de-regressed bull EBV); μ is the population mean; b is a vector of SNP fitted as a fixed effect; a is a vector of additive polygenic random effect including the genomic relationship matrix among individual animals; X and Z are incidence matrices for fixed effects and random effects, respectively; and e is the vector of residuals.

GWA analyses were conducted with the R-based statistical package GenABEL [28]. After Bonferroni correction, the genome-wide significant threshold (P = 0.05) was defined at P = 1.43 x 10^{-6} which corresponds to a $-\log_{10}$ (P) = 5.84, whereas the suggestive threshold (i.e. one false positive per genome scan) was defined at P = 2.87 x 10^{-5} corresponding to a $-\log_{10}$ (P) = 4.54. The P-values obtained from the GWA analysis were adjusted for inflation using the genomic inflation factor, λ, which accounts for any systematic deviation of observed from expected P-values. The estimated polygenic heritability was calculated as $h^2 = (\sigma^2_a / \sigma^2_p)$ in which the phenotypic variance (σ^2_p) was obtained by summing the additive genetic (σ^2_a) and residual variance (σ^2_e) from model 1.

SNPs found to be significant in the previous step were further tested by fitting the respective genotypes individually as a fixed effect in a mixed model similar to model 1. These analyses were conducted with the ASReml software package [29]. The genotypic effect solutions were used to estimate the additive and dominance effects of

the respective loci. The proportion of genetic variance of each trait explained by each SNP was estimated using the following equation:

Proportion of genetic variance explained by SNP = $[2pq\ (a+d\ (q-p))^2]/\ \sigma^2_a$

where a, d, p and q were respectively additive effects, dominance effects, allele frequencies at the SNP locus and σ^2_a is the total genetic variance of the trait calculated with model 1 excluding the SNP effect.

Significant SNPs were also explored for linkage disequlibrium (LD) with other nearby SNPs. Pairwise LD, measured with r^2 was calculated in the software PLINK [25] with LD and haplotype blocks visualised in Haploview software [30]. The haplotype blocks were identified using Wang's method [31]. QTL regions surrounding significant SNPs were defined by the farthest neighbouring SNPs that had a minimum LD of 0.40 with the significant SNP in question. Subsequently, in order to identify candidate genes, the QTL regions were then matched onto the bovine reference genome that is publicly available through the *Bos_taurus_UMD_3.1.1* project of the National Centre for Biotechnology Information [32].

2.2.3.4 Regional heritability mapping

The same data described above were analysed with the RHM approach, in which genomic regions of 100 SNPs were defined by sliding 'windows' shifting every 50 SNPs along each autosomal chromosome. A detailed description of RHM was given by [33].

The following model was applied for the RHM:

$$y = \mu + Xb + Za + Zr + e \qquad (2)$$

where r is a vector of region (consisting of 100 SNPs) fitted as a random effect; with other terms in the model defined as in model (1).

RHM analyses were performed using the DISSECT software [34]. The significance of genomic regions was assessed with the likelihood ratio test (LRT) statistic, which was used to compare model (2) that fitted a genomic region as a random effect against the base model that excluded this effect. The LRT was derived as twice the difference between the log-likelihoods of the model including and excluding the regions in question. A total of 713 regions were tested across the genome, of which half were used in the Bonferroni correction to account for the shifting of regions every 50 SNPs. The LRT thresholds were 13.20 ($P = 1.40 \times 10^{-4}$) and 8.93 ($P = 2.80 \times 10^{-3}$) for the genome-wide and suggestive significance thresholds, respectively. The phenotypic variance was calculated as $\sigma^2_p = \sigma^2_a+\sigma^2_r+\sigma^2_e$, while the regional (r) heritability was subsequently estimated as $h^2_r = \sigma^2_r/\sigma^2_p$.

2.2.3.5 Chromosome association analysis

In a separate set of analyses, the entire autosomal chromosome effect was fitted in model 2 instead of genomic region. After Bonferroni correction, the LRT significance thresholds for the genome-wide and suggestive levels were 8.55 ($P = 1.72 \times 10^{-3}$) and 4.47 ($P = 3.45 \times 10^{-2}$), respectively. The phenotypic variance was calculated as $\sigma^2_p = \sigma^2_a+\sigma^2_c+\sigma^2_e$, where σ^2_c was the variance due to the chromosomal genetic effect. The chromosomal (c) heritability was subsequently estimated as $h^2_c =\sigma^2_c/\sigma^2_p$.

2.2.4 Results

The multi-dimensional scaling analysis indicated that the sample population was homogenous, manifested by a single cluster of individuals (Additional file 2.1). The mean de-regressed EBVs for susceptibility to bTB among the traits ranged from 0.38 to 0.47 with mean reliabilities of de-regressed EBVs ranging between 0.69 and 0.74

(Additional file 2.2). Correlation between sire de-regressed EBVs was high between phenotypes 2 and 3 (0.99), and lower between phenotypes 1 and 2 (0.54) and between phenotypes 1 and 3 (0.57).

2.2.4.1 GWA analysis

Association between individual SNPs and bTB susceptibility traits are illustrated in the Manhattan plots in Figure 2.1, with corresponding quantile-quantile plots in Additional file 2.3. Estimated polygenic heritability for the three bTB traits was moderate and ranged from 0.26 ± 0.07 to 0.37 ± 0.07, with heritabilities for phenotypes 2 and 3 being similar but both a little higher than for phenotype 1 (Additional file 2.2).

We identified three suggestive SNPs associated with the studied traits (Table 2.1). Two of these SNPs, ARS-BFGL-NGS-40833 ($P = 2.56 \times 10^{-5}$) and Hapmap38114-BTA-57971 ($P = 1.48 \times 10^{-5}$) were associated with phenotype 1 on BTA 2 and 24, respectively. The other SNP, BTA-56563-no-rs ($P = 1.99 \times 10^{-5}$) on BTA 23 was associated with phenotype 2. The SNP identified to affect phenotype 2 also reached but did not exceed the suggestive significance threshold for phenotype 3 (Figure 2.1).

Additive and dominance effects of these SNPs and the proportion of the genetic variance explained by them are shown in Additional file 2.4. SNPs on BTA 2 and 23 had significant ($P<0.01$) additive effects on phenotypes 1 and 2, respectively. However, there was no significant additive effects found for the SNP on BTA 24. The additive (allele substitution B to A) effect for the SNP on BTA 2 was 0.57 and the SNP accounted for 14% of the total genetic variance of susceptibility to bTB as defined by phenotype 1.

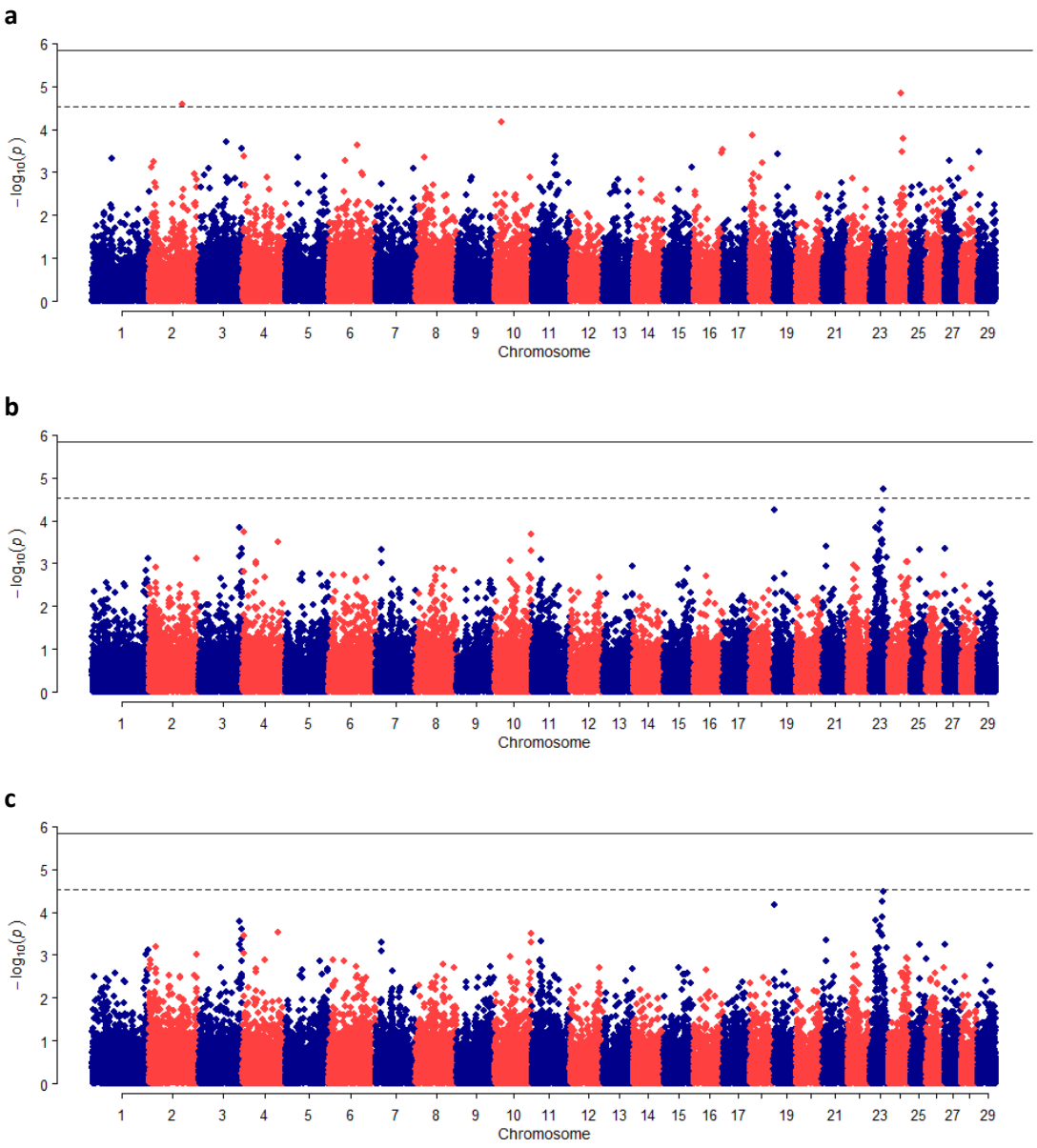


Figure 2.1 Manhattan plots displaying results of genome-wide association analyses of three bovine tuberculosis susceptibility traits: (a) phenotype 1, positive reactors to the skin test with positive post-mortem results; (b) phenotype 2, positive reactors to the skin test regardless of post-mortem results; (c) phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive post-mortem examination results. Dashed and solid lines represent suggestive and genome-wide thresholds, respectively.

The SNP on BTA 23 had an additive (allele substitution B to A) effect of 0.81 and explained 3% of the genetic variance of susceptibility to bTB as defined by phenotype 2. In both cases, the minor allele A was associated with increased resistance

to bTB infection. No significant dominance effects ($P > 0.05$) were found for any SNP locus.

Table 2.1 SNPs identified in the genome-wide association analysis to be significantly associated with bovine tuberculosis traits. Phenotype 1, positive reactors to the skin test with positive post-mortem results; phenotype 2, positive reactors to the skin test regardless of post-mortem results.

Phenotype	SNP name	BTA	Position	P-value
1	ARS-BFGL-NGS-40833	2	93065483	2.56×10^{-5}
	Hapmap38114-BTA-57971	24	35403612	1.48×10^{-5}
2	BTA-56563-no-rs	23	38412668	1.99×10^{-5}

Putative QTL regions were defined based on the LD of our two significantly additive SNPs with neighbouring SNPs. The LD structure for these regions is presented in Additional files 2.5 and 2.6 for SNPs on BTA 2 and 23, respectively. The SNP on BTA 2 was located within a QTL region spanning 1.29 Mb. One relevant gene in the bovine reference genome found within this region, *PARD3B*, was about 157 Kb upstream of the SNP. The SNP identified on BTA 23 was located within a QTL region covering 1.2 Mb. The most relevant gene found in the region was *RNF144B,* located upstream of BTA-56563-no-rs.

Overall, the GWA analysis results showed that, although some SNPs are significantly associated with the traits of study, a considerable proportion of the genetic variance still remains unaccounted for. This is expected for traits with largely complex polygenic architectures.

2.2.4.2 RHM analysis

The RHM analysis revealed two regions that crossed the genome-wide significance threshold for phenotypes 2 and 3 on BTA23 (Table 2.2; Figure 2.2). Additional regions reached the suggestive significance threshold on BTA 3, 18 and 23 across the three traits (Table 2.2; Figure 2.2).

Three overlapping regions were identified on BTA 23 affecting both phenotype 2 and 3: region 1 (30.2 - 38.4 Mb), region 2 (33.9 - 41.6 Mb) and region 3 (38.5 - 44.8 Mb). The SNP identified on BTA 23 with the GWA analysis was located within regions 1 and 2. The regional heritability estimates ranged from 0.05 to 0.08 (Table 2.2).

Two new significant regions on BTA 3 and 18, associated with phenotypes 2 and 3, and phenotype 1, respectively, were revealed. The GWA analysis had not identified any significant SNPs in these regions. Corresponding regional heritability estimates ranged between 0.06 and 0.08 (Table 2.2).

Another region on BTA 24 associated with phenotype 1, within which the SNP identified with the GWA analysis had been located, was just below the suggestive threshold of RHM (Figure 2.2).

2.2.4.3 Chromosome association analysis

The chromosomal association study (Additional file 2.7) revealed that BTA 23 had the greatest impact on phenotypes 2 and 3, and the highest LRT of 15.88 and 15.26, respectively. This is consistent with the GWA and RHM results. Corresponding chromosomal heritability estimates were 0.07 ± 0.03 and 0.08 ± 0.04 for the two traits, suggesting the regions identified with RHM in the present study were entirely responsible for this chromosome's effect.

Table 2.2 Genomic regions identified with regional heritability mapping (100-SNP windows) affecting three bovine tuberculosis traits. Phenotype 1, positive reactors to the skin test with positive post-mortem results; phenotype 2, positive reactors to the skin test regardless of post-mortem results; phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive post-mortem examination results.

Phenotype	BTA	Genomic regions (SNP name and position (bp))		LRT	h^2_r (SE)
		Start	End		
1	18	Hapmap57004-rs29011610 4463083	ARS-BFGL-NGS-1116 9539002	9.41*	0.06 (0.03)
2	23	ARS-BFGL-NGS-78313 30222836	BTA-56563-no-rs 38412668	15.12#	0.05 (0.03)
	23	ARS-BFGL-NGS-107881 33961556	BTB-00870908 41672507	20.41#	0.07 (0.03)
	23	Hapmap31420-BTA-137383 38521106	ARS-BFGL-NGS-41732 44897933	9.27*	0.05 (0.03)
	3	ARS-BFGL-84593 114388249	ARS-BFGL-NGS-26427 119113936	9.48*	0.07 (0.03)
3	23	ARS-BFGL-NGS-78313 30222836	BTA-56563-no-rs 38412668	15.98#	0.05 (0.03)
	23	ARS-BFGL-NGS-107881 33961556	BTB-00870908 41672507	21.37#	0.08 (0.03)
	23	Hapmap31420-BTA-137383 38521106	ARS-BFGL-NGS-41732 44897933	9.76*	0.05 (0.03)
	3	ARS-BFGL-84593 114388249	ARS-BFGL-NGS-26427 119113936	10.37*	0.08 (0.04)

#Genome-wide significance level; *Suggestive significance level; h^2_r = regional heritability; SE = standard error.

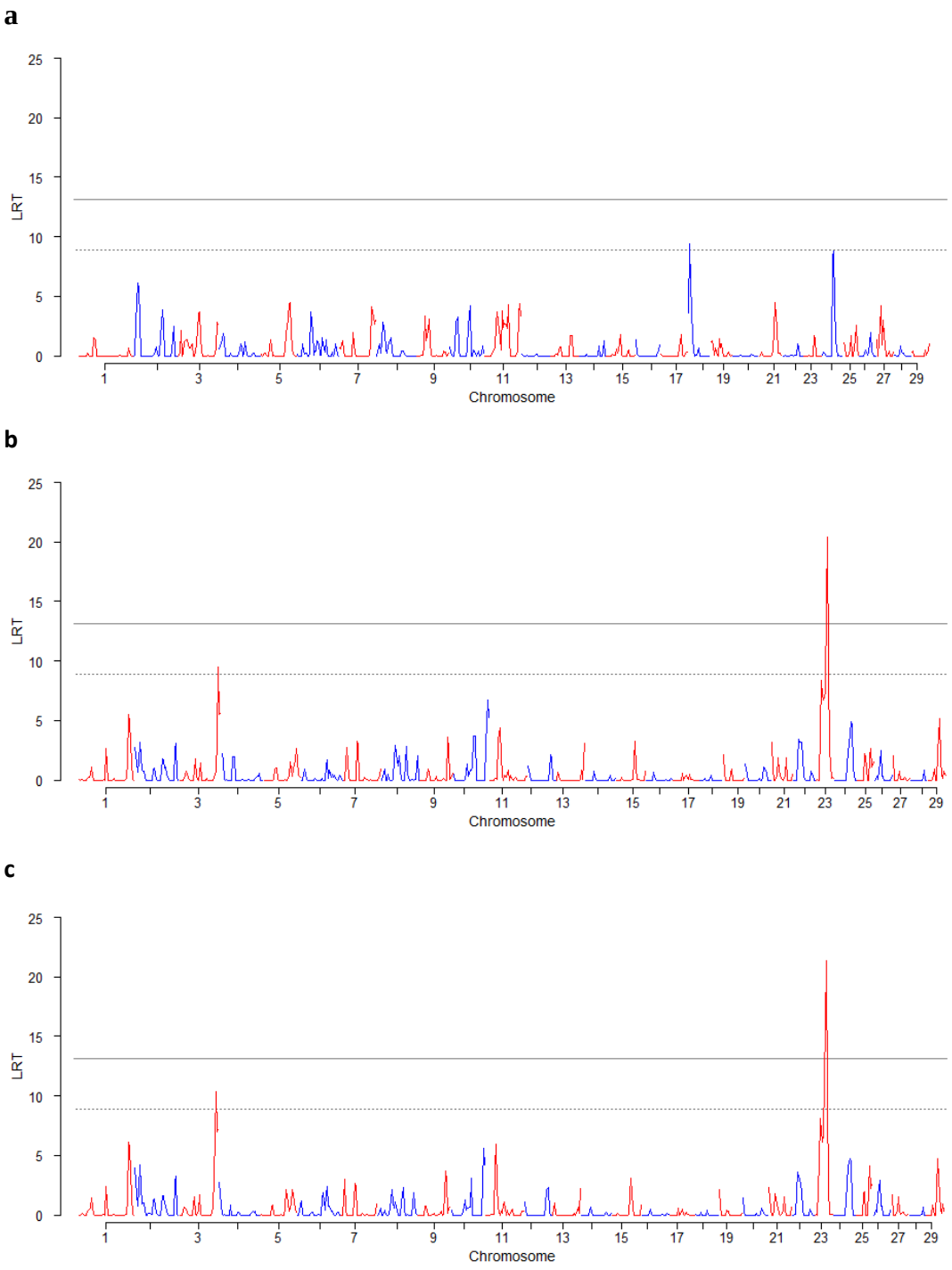


Figure 2.2 Manhattan plots displaying results of regional heritability mapping analyses of three bovine tuberculosis susceptibility traits. (a) phenotype 1, positive reactors to the skin test with positive post-mortem results; (b) phenotype 2, reactors to the skin test regardless of post-mortem results; (c) phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive post-mortem examination results. Dashed and solid lines represent suggestive and genome-wide thresholds, respectively.

Regarding phenotype 1, the highest significant LRT was observed on a different chromosome (BTA 11), where neither GWA nor RHM analyses had revealed any significant associations. The corresponding chromosomal heritability was 0.08 ± 0.04 and was probably due to an aggregation of moderate effects of different genomic regions along this chromosome. Similarly, neither BTA 18 nor BTA 24, where RHM had revealed genomic regions with suggestive effects, reached a significance level in the chromosomal association analysis of phenotype 1 (Additional file 2.7).

2.2.5 Discussion

Our results offer insights into the genomic architecture of susceptibility to bTB in British Holstein-Friesian dairy cattle. This is the first genomic study of this population that explores three different case phenotypes based on the bTB testing regime undertaken in Great Britain. In all cases, we used de-regressed sire EBVs as phenotypes. The latter are considered robust phenotypes for genomic analyses [23, 24, 35], representing the aggregate adjusted records for disease incidence of multiple progeny per sire.

The findings of the present study collectively suggest that considerable heritable variation at the genomic level influences differences in the inherent bTB susceptibility among animals. We found that heritability for bTB susceptibility was moderately high in this population and therefore selection for resistance is a feasible strategy to reduce the incidence of bTB nationwide. Other studies [12-16] corroborate these findings. Tsairidou et al. [13] and Bermingham et al. [19], respectively reported polygenic heritabilities of 0.23 and 0.21 for susceptibility to bTB, which were similar to the estimate for phenotype 1 in our study, based on positive skin test reactors with positive post mortem examination results. However, these heritability estimates were

lower than those obtained for phenotypes 2 and 3 in the present study; these two trait definitions account for skin test imperfections and therefore, are likely to represent a different phenotype compared to conventionally confirmed cases. This finding is further supported by the relatively lower correlations between sire EBVs for phenotype 1 and those of the other two traits, which are in agreement with results from Banos et al. [15].

GWA analysis conducted in the present study identified two QTL regions that may influence animal susceptibility to bTB. The global Holstein-Friesian cattle population has high levels of genetic relatedness among animals (population structure) manifested by a small effective population size, which may result in false associations [36]. However, in the present study, inclusion of the genomic relationship matrix in the model accounted for the population structure. Relatively few individual SNPs with a significant effect on the bTB traits were identified through GWA analysis. This could be explained by the complex genetic architecture underlying susceptibility to bTB and the polygenic nature of the disease as suggested by Bermingham et al. [19]. It could also be partly attributed to the conservativeness of the Bonferroni correction method used to adjust for multiple testing, which often inflates type II errors [37].

The present study identified two additive SNPs in moderate LD with neighbouring SNPs on BTA 2 and 23 that were significantly associated with different traits of susceptibility to bTB. In both cases, the allele with the minor frequency had the favourable additive effect, conferring increased resistance to bTB in the studied population. A similar result reported by Bermingham et al. [19] indicated that the major frequency alleles of SNPs on BTA 2 (different region compared to our study) and 13 were associated with a greater risk of bTB infection. Richardson et al. [18],

however, found that the major frequency alleles of SNPs located on BTA 1 and 23 (different region compared to our study) were associated with bTB resistance. In all cases, different SNPs and cattle populations are involved. The SNPs identified in the present study provide possible markers for selecting against susceptible individuals with the potential to improve inherent resistance to the disease in the British Holstein population.

The length of the putative QTL regions defined in the present study (1.20-1.29 Mb) was similar to those reported by Kim and Kirkpatrick [38] where the median physical distance between pairs of markers at a mean LD of 0.48 was about 1.13 Mb in Holstein cattle. We identified candidate genes within these regions with possible underlying effects on disease susceptibility. The significant SNP on BTA 2 was located close to gene *PARD3B*, which has been implicated in protection against disease progression in patients affected by the human immune deficiency virus and acquired immune deficiency syndrome (HIV/AIDS) [39]. Similarly to bTB in cattle, HIV/AIDS is a chronic, progressive illness of humans. The most relevant gene close to the SNP on BTA 23 was *RNF144B*. This protein coding gene has been found to play a role in the regulation of NF-κB in human macrophages. NF-κB regulates the expression of various genes involved in diverse cellular processes including inflammation and immunity [40] and has been associated with endometriosis in humans [41]. Other functions of the *RNF144B* gene include roles in regulation of apoptosis and cell proliferation, making the gene a possible candidate for therapeutic treatment of endometrial cancer [42]. Further studies based on expression profiles and pathway analyses may shed more light into the function of the above genes in relation to cattle susceptibility to bTB.

The present study did not confirm QTL identified in previous association studies on bTB susceptibility [17-21], which further supports the notion of a polygenic trait controlled by multiple genes. The closest GWA results on BTA 23 were reported by Richardson et al. [18] who identified a QTL about 28 Mb downstream on the same chromosome for Irish dairy cattle. Richardson et al. [18] also used de-regressed EBVs based on a phenotype similar to phenotype 2 in our study.

The RHM analysis overcame some of the limitations of GWA due to the former's capacity to consolidate a proportion of genomic variation based on multiple neighbouring marker effects [33]. In the present study, RHM identified significant new genomic regions on BTA 18 for phenotype 1 and BTA 3 for phenotypes 2 and 3, where GWA had not identified individual SNPs with a significant effect on the respective traits. This suggests that RHM may identify regions harbouring individual SNPs with moderate or even non-significant effects, which, however, may collectively have a significant impact on bTB susceptibility. Importantly, RHM also identified significant genomic regions including the individual SNPs with a significant effect in the GWA analysis, thereby corroborating the suggestion of a QTL presence. The three genomic regions identified on BTA 23 support the possibility of a large region with overlapping genetic variants. RHM has previously been used in association studies of susceptibility to bTB in a different cattle population [19, 43]. Although no common regions with those of our study were reported, Wilkinson et al. [43] identified a region further downstream (at 6.6 - 7.1 Mb) of our region on BTA 23 affecting positive reactors to the skin test with negative post-mortem results (unconfirmed cases).

Furthermore, the present study has highlighted a major overall chromosomal influence of BTA 23 on susceptibility to bTB, when the definition of the latter is not

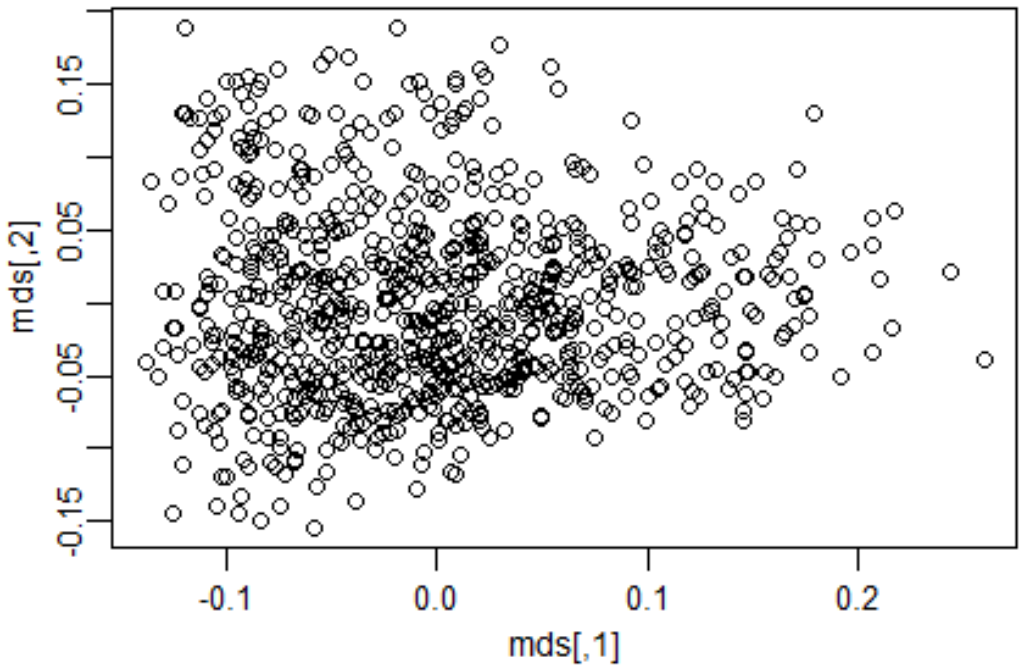
restricted to post-mortem confirmed cases but includes all positive skin test reactors and all animals with a positive post-mortem result. Actually, chromosome 23 was the only chromosome that featured in the significant results of all our analyses (GWA, RHM, chromosomal association). Notably, BTA 23 harbours the major histocompatibility complex (MHC), which plays a central role in immune response to infection [44, 45]. Our region was located about 10 Mb upstream of the MHC region based on GWA and 2 Mb based on RHM results. In addition, Zare et al. [46] found genomic regions on BTA 23 (at 35.3 and 44.4 Mb) associated with paratuberculosis in Jersey cattle, a disease with certain similarities to bTB. These regions corresponded to our RHM identified regions on BTA 23.

Previous genomic studies on cattle susceptibility to bTB have not resulted in consistent outcomes to support a common genomic mechanism underlying the trait. Some of our results might have added to the wealth of diverse findings. As discussed, reasons for such discrepancies include the complexity of the phenotype, the largely polygenic inheritance mode of the trait, genetic differences between populations and differences in methodologies used across studies. Additional reasons may be different allele frequencies of either the marker or causative mutation even when the same QTL is segregating in various populations, and possible mutation linkage phases that may not be the same between populations [20, 47]. Moreover, bTB is an infectious disease whose profile and transmission dynamics may differ across populations and geographic regions, thereby further complicating the genomic study of the underlying control mechanism. All these reasons together suggest that scientific results are likely to be relevant primarily to the studied population and trait definitions on which they were based.

2.2.6 Conclusions

Our results suggest that bTB susceptibility in the British Holstein cattle population is a moderately heritable polygenic trait, potentially amenable to improvement with selective breeding. Our findings may inform genomic predictions (genomic EBV calculations) within a genomic selection programme, where differential emphasis can be placed on specific genomic regions identified to have significant effects on the trait. At the same time, it would be useful to quantify the impact of such a selection process on the disease dynamics as well as other traits of the breeding goal. Our results may also provide target areas for possible future gene editing applications within a genetic improvement programme.

2.2.7 Additional files



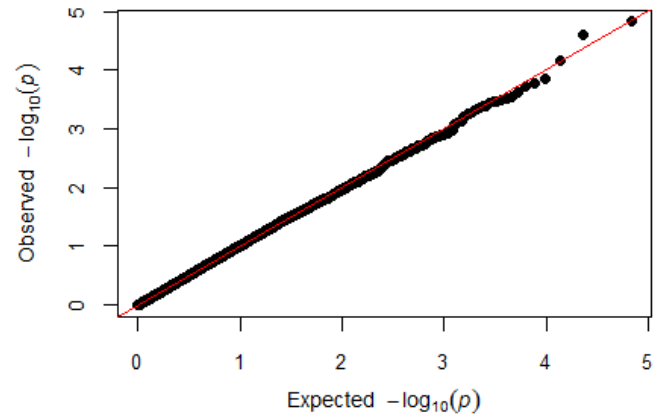
Additional file 2.1 Multi-dimensional scaling (Principal Component) analysis of an identity by state matrix of 804 bulls. A single cluster was formed which reflect homogeneity of the population.

Additional file 2.2 Genetic parameters of three bovine tuberculosis traits

Phenotype[1]	Mean de-regressed EBV	Mean reliability of sire EBV	Polygenic heritability (SE)
1	0.38	0.69	0.26 (0.07)
2	0.44	0.74	0.37 (0.07)
3	0.47	0.74	0.34 (0.07)

[1]Phenotype 1, positive reactors to the skin test with positive post-mortem results; phenotype 2, positive reactors to the skin test regardless of post-mortem results; phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive
post-mortem examination results
EBV = estimated breeding value; SE = standard error

a



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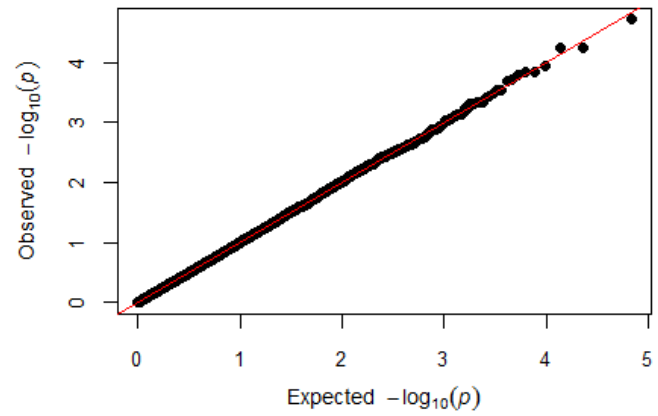

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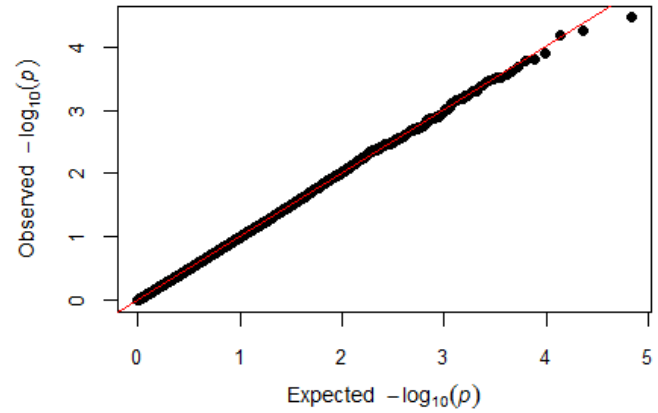


Additional file 2.3 Quantile-quantile plots of observed against expected P-values from genome-wide association analyses. (a) phenotype 1, positive reactors to the skin test with positive post-mortem results; (b) phenotype 2, positive reactors to the skin test regardless of post-mortem results; (c) phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive post-mortem examination results.

Additional file 2.4 Additive and dominance effects for significant SNPs identified by genome-wide association analysis.

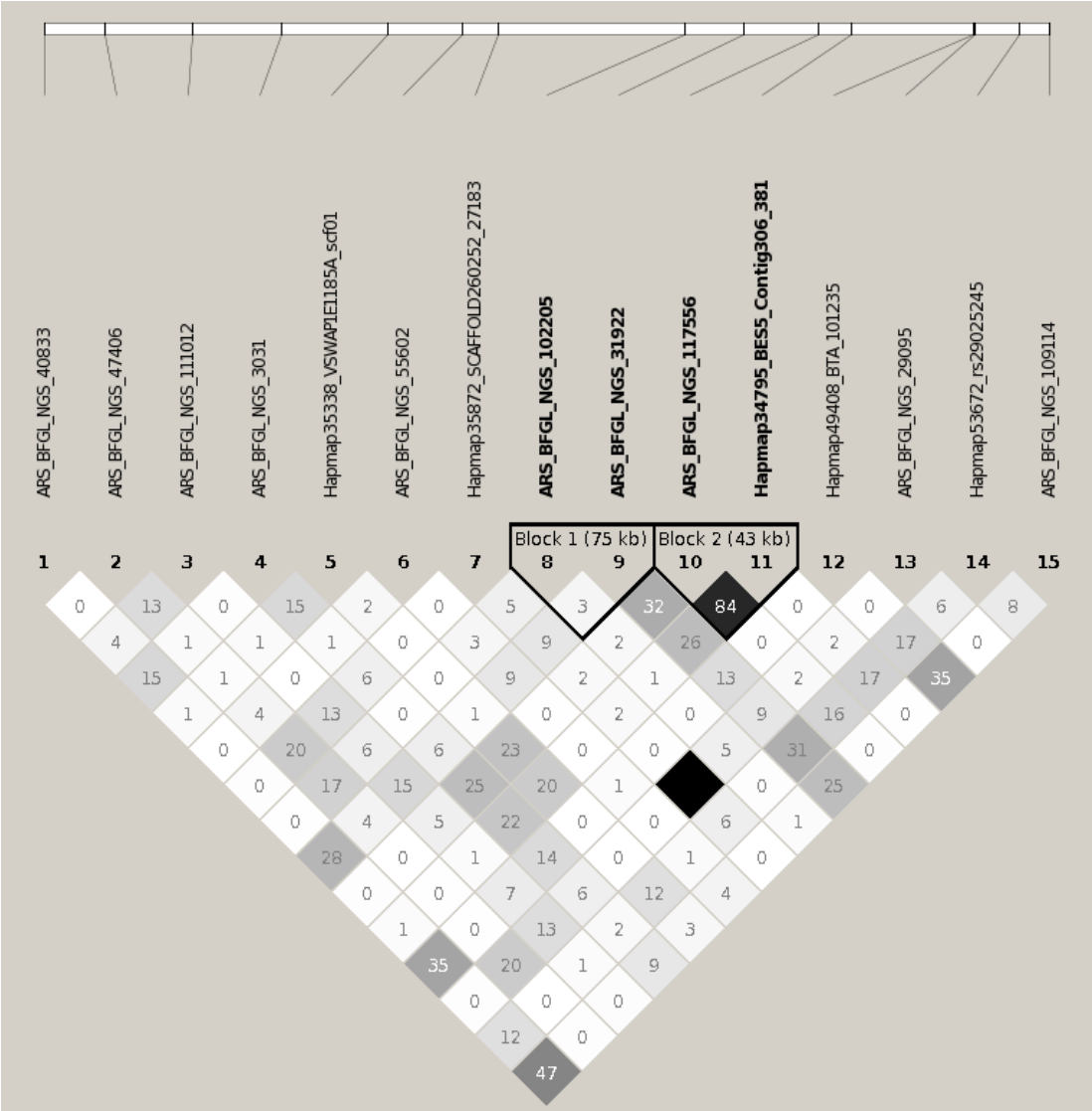
Phenotype[1]	SNP	Allele frequency		P-value (SNP)	a (SE)	P-value (a)	d (SE)	P-value (d)	VA_{prop}
		p	q						
1	SNP1	0.63	0.37	0.001	0.57 (0.14)	$7.74x10^{-5}$	-0.05 (0.17)	0.38	0.14
	SNP2	0.89	0.11	0.001	0.66 (0.36)	$7.90x10^{-2}$	0.23 (0.40)	0.34	0.04
2	SNP3	0.84	0.16	0.001	0.82(0.32)	$1.57x10^{-2}$	0.31 (0.36)	0.27	0.03

[1]Phenotype: phenotype 1: positive reactors to the skin test with positive post-mortem results; phenotype 2, all reactors to the skin test regardless of post-mortem results.

Allele frequency: p and q; a = additive genetic effect; d = dominance effect; SE = standard error;

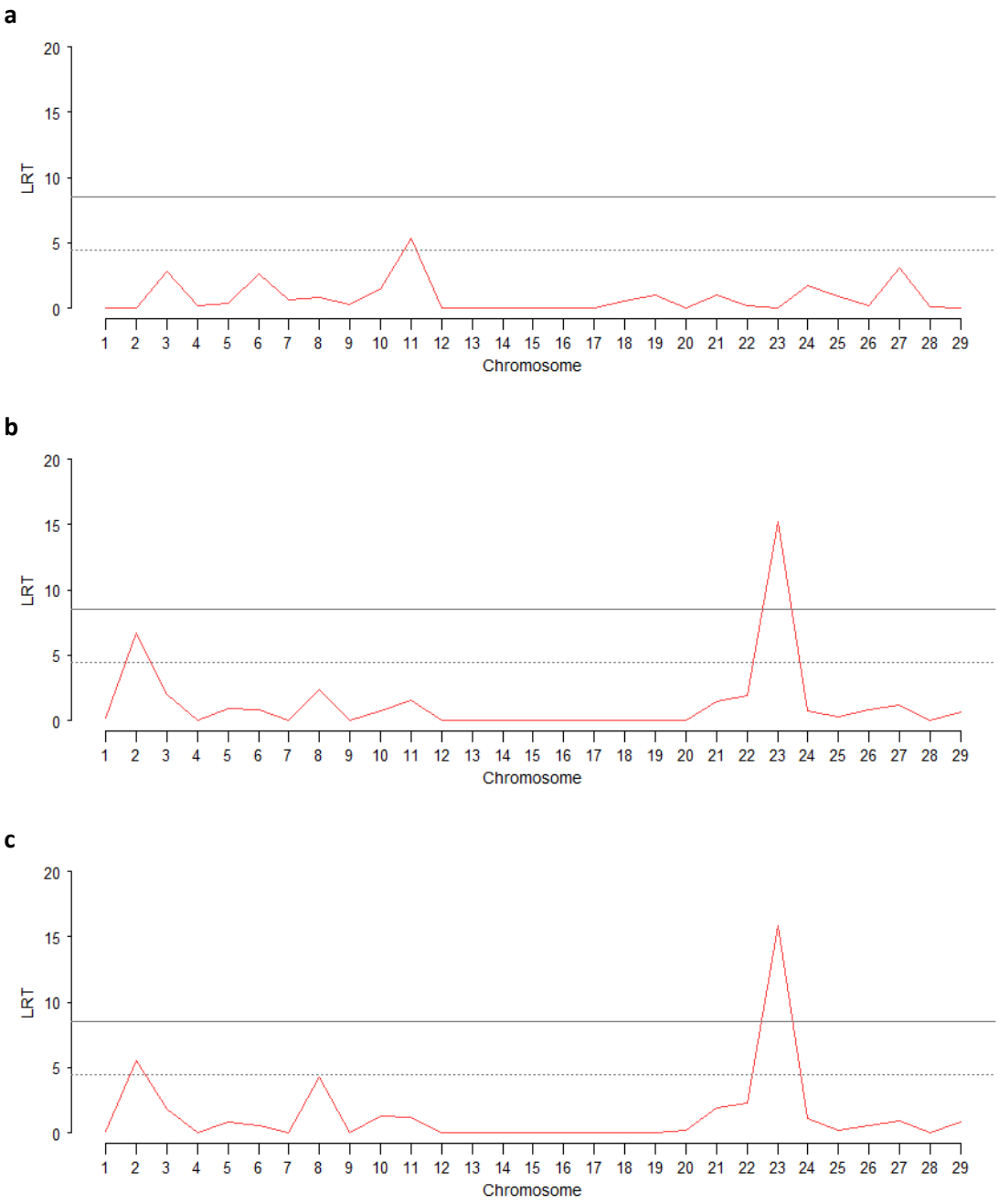
VA_{prop} = proportion of genetic variance due to SNP, where VA is the total additive genetic variance estimated from a model ignoring SNP effects;

SNP1 = ARS-BFGL-NGS-40833; SNP2= Hapmap38114-BTA-57971; SNP3 = BTA-56563-no-rs.



Additional file 2.5 Linkage disequilibrium (r^2) map of a QTL region on BTA 2 affecting bTB (phenotype 1). The region ranges from SNP ARS-BFGL-NGS-40833 (bp = 93065483) to SNP ARS-BFGL-NGS-109114 (bp = 94352603); white for $r^2 = 0$, shades of grey for $0 < r^2 < 1$ and black for $r^2 = 1$.

Additional file 2.6 Linkage disequilibrium (r^2) map of a QTL region on BTA 23 affecting bTB (phenotype 2). The region ranges from SNP ARS-BFGL-NGS-88425 (bp = 38206814) to SNP BTA-01409-rs29012374 (bp = 39411428); white for $r^2 = 0$, shades of grey for $0 < r^2 < 1$ and black for $r^2 = 1$.



Additional file 2.7 Manhattan plots displaying results of chromosomal association analyses of three bovine tuberculosis susceptibility traits: (a) phenotype 1, positive reactors to the skin test with positive post-mortem results; (b) phenotype 2, positive reactors to the skin test regardless of post-mortem results; (c) phenotype 3, as phenotype 2 plus non-reactors and inconclusive reactors with positive post-mortem examination results. Dashed and solid lines represent suggestive and genome-wide thresholds, respectively.

2.2.8 Abbreviations

SNP, single neucleotide polymorphism; bTB, bovine tuberculosis; EBV, estimated breeding value; BTA, *Bos taurus* autosome; GWA, genome-wide association; RHM, regional heritability mapping; LRT, likelihood ratio test; QTL, quantitative trait loci; LD, linkage disequilibrium.

2.2.9 Declarations

***Acknowledgements*:** Data for this study (estimated breeding values and genotypes of individual animals) were made available by the Edinburgh Genetic Evaluation Services (EGENES) within Scotland's Rural College (SRUC) following agreement with the Animal and Plant Health Agency (APHA) and the Agriculture and Horticulture Development Board (AHDB-Dairy).

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***Availability of data and material*:** Background data that support the findings of this study are part of the British national genetic evaluations on bovine tuberculosis conducted by EGENES on behalf of the dairy cattle industry (AHDB-Dairy). Any request for data and material will be addressed in conjunction with AHDB-Dairy.

***Authors' contributions*:** EJG, JAW, SCB, RM, MC and GB participated in the design and sourcing of funding for the encompassing project on selection for bovine

tuberculosis resistance. KR, OM and GB prepared the manuscript. KR, OM, ESM, VR and GB were responsible for data editing, analysis and interpretation of results. MC, RM and GB generated phenotypic data (sire de-regressed EBVs) and availed genomic data for this study. ESM, VR, EJG, JAW, RM and MC revised the manuscript and improved its content. All authors have read and approved the manuscript.

***Competing interests*:** The authors declare that they have no competing interests.

***Consent for publication*:** Not applicable

***Ethics approval and consent to participate*:** Data were sire genetic values and genotypes that had been generated prior to the present study; specifically, these data were from the official national genetic and genomic evaluations run at SRUC (ref. in manuscript: [15, 22]) and were made available to the present study under a Material Transfer Agreement between SRUC and the University of Edinburgh (signed on 7, April 2015). No individual animal records were used for this study.

2.2.10 References

1. Green LE, Cornell SJ. Investigations of cattle herd breakdowns with bovine tuberculosis in four counties of England and Wales using VETNET data. Prev Vet Med. 2005; 70(3–4):293-311.
2. De la Rua-Domenech R. Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis. 2006; 86(2):77-109.
3. Lawes J, Harris K, Brouwer A, Broughan J, Smith N, Upton P. Bovine TB surveillance in Great Britain in 2014. Vet Rec. 2016; 178(13):310-315.
4. Johnston WT, Vial F, Gettinby G, Bourne FJ, Clifton-Hadley RS, Cox DR, et al. Herd-level risk factors of bovine tuberculosis in England and Wales after

the 2001 foot-and-mouth disease epidemic. Int J Infect Dis. 2011; 15(12):E833-E840.

5. Abernethy DA, Upton P, Higgins IM, McGrath G, Goodchild AV, Rolfe SJ, et al. Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995-2010. Vet Rec. 2013; 172(12):312.

6. Department for Environment, Food and Rural Affairs. Incidence of TB in cattle in Great Britain. https://www.gov.uk/government/uploads/system/ https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/372947/bovinetb-statsnotice-12nov14.pdf. (Accessed 16th July 2016).

7. De la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci. 2006; 81(2):190-210.

8. Allen AR, Minozzi G, Glass EJ, Skuce RA, McDowell SWJ, Woolliams JA, Bishop SC. Bovine tuberculosis: the genetic basis of host susceptibility. Proc R Soc Lond B Biol Sci. 2010; 277(1695):2737-2745.

9. Vial F, Miguel E, T Johnston W, Mitchell A, Donnelly C. Bovine Tuberculosis risk factors for British herds before and after the 2001 foot-and-mouth epidemic: What have we learned from the TB99 and CCS2005 studies? Transbound Emerg Dis. 2015; 62(5):505-515.

10. Bessell PR, Orton R, White PC, Hutchings MR, Kao RR. Risk factors for bovine tuberculosis at the national level in Great Britain. BMC Vet Res. 2012; 8(1):1.

11. Karolemeas K, McKinley TJ, Clifton-Hadley RS, Goodchild AV, Mitchell A, Johnston WT, Conlan AJK, Donnelly CA, Wood JLN. Recurrence of bovine tuberculosis breakdowns in Great Britain: Risk factors and prediction. Prev Vet Med. 2011; 102(1):22-29.

12. Brotherstone S, White I, Coffey M, Downs S, Mitchell A, Clifton-Hadley R, More S, Good M, Woolliams J. Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*. J Dairy Sci. 2010; 93(3):1234-1242.

13. Tsairidou S, Woolliams JA, Allen AR, Skuce RA, McBride SH, Wright DM, et al. Genomic prediction for tuberculosis resistance in dairy cattle. PLoS One. 2014; 9(5):e96728.

14. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Brotherstone S, Berry DP. Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. J Dairy Sci. 2009; 92(7):3447-3456.

15. Banos G, Winters M, Mrode R, Mitchell A, Bishop SC, Woolliams JA, Coffey MP. Genetic evaluation for bovine tuberculosis resistance in dairy cattle. J Dairy Sci. 2016; 100(2):1272-1281.

16. Richardson IW, Bradley DG, Higgins IM, More SJ, McClure J, Berry DP. Variance components for susceptibility to *Mycobacterium bovis* infection in dairy and beef cattle. Genet Sel Evol. 2014; 46(1):1.

17. Finlay EK, Berry DP, Wickham B, Gormley EP, Bradley DG. A genome wide association scan of bovine tuberculosis susceptibility in Holstein-Friesian dairy cattle. PLoS One. 2012; 7(2):e30545.

18. Richardson IW, Berry DP, Wiencko HL, Higgins IM, More SJ, McClure J, Lynn DJ, Bradley DG. A genome-wide association study for genetic

susceptibility to *Mycobacterium bovis* infection in dairy cattle identifies a susceptibility QTL on chromosome 23. Genet Sel Evol. 2016; 48(1):1.

19. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, et al. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity. 2014; 112(5):543-551.

20. Tsairidou S, Woolliams J, Allen A, Skuce R, McBride S, Pong-Wong R, et al. A meta-analysis for bovine tuberculosis resistance in dairy cattle. Adv Anim Biosci. 2015:68.

21. Kassahun Y, Mattiangeli V, Ameni G, Hailu E, Aseffa A, Young DB, Hewinson RG, Vordermeier HM, Bradley DG. Admixture mapping of tuberculosis and pigmentation-related traits in an African-European hybrid cattle population. Front Genet. 2015; 6:210.

22. Mrode R. Application of various models for the genomic evaluation of bovine tuberculosis in dairy cattle. In: Proc 2016 Interbull Annual Meeting, Puerto Varas, Chile. 2016.

23. Ekine CC, Rowe SJ, Bishop SC, de Koning D-J. Why breeding values estimated using familial data should not be used for genome-wide association studies. G3: Genes| Genomes| Genetics. 2013; g3.113.008706.

24. Garrick DJ, Taylor JF, Fernando RL. Deregressing estimated breeding values and weighting information for genomic regression analyses. Genet Sel Evol. 2009; 41(1):1.

25. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559-575.

26. Amin N, Van Duijn CM, Aulchenko YS. A genomic background based method for association analysis in related individuals. PloS one. 2007; 2(12):e1274.

27. Jensen J, Su G, Madsen P. Partitioning additive genetic variance into genomic and remaining polygenic components for complex traits in dairy cattle. BMC Genet. 2012; 13:44.

28. Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM. GenABEL: an R library for genome-wide association analysis. Bioinformatics. 2007; 23(10):1294-1296.

29. Gilmour AR, Gogel B, Cullis B, Thompson R, Butler D. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, UK. 2009.

30. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263-265.

31. Wang N, Akey JM, Zhang K, Chakraborty R, Jin L. Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. Am J Hum Genet. 2002; 71(5):1227-1234.

32. National Centre, for Biotechnology Information. Genome: *Bos taurus*. http://www.ncbi.nlm.nih.gov/genome/82. Accessed 5th August 2016.

33. Nagamine Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Rudan I, et al. Localising loci underlying complex trait variation using regional genomic relationship mapping. PLoS One. 2012; 7(10):e46501.

34. Canela-Xandri O, Law A, Gray A, Woolliams JA, Tenesa A. A new tool called DISSECT for analysing large genomic data sets using a big data approach. Nat Commun. 2015; 6.

35. Ostersen T, Christensen OF, Henryon M, Nielsen B, Su G, Madsen P. Deregressed EBV as the response variable yield more reliable genomic predictions than traditional EBV in pure-bred pigs. Genet Sel Evol. 2011; 43(1):1.

36. Astle W, Balding DJ. Population structure and cryptic relatedness in genetic association studies. Stat Sci. 2009; 24(4):451-471.

37. Johnson RC, Nelson GW, Troyer JL, Lautenberger JA, Kessing BD, Winkler CA, O'Brien SJ. Accounting for multiple comparisons in a genome-wide association study (GWAS). BMC Genomics. 2010; 11(1):1.

38. Kim ES, Kirkpatrick B. Linkage disequilibrium in the North American Holstein population. Anim Genet. 2009; 40(3):279-288.

39. Troyer JL, Nelson GW, Lautenberger JA, Chinn L, McIntosh C, Johnson RC, et al. Genome-wide association study implicates PARD3B-based AIDS restriction. J Infect Dis. 2011; 203(10):1491-1502.

40. Baek S-H, Huang B, Chang HW. RNF144b is a negative regulator in TLR2-mediated NF-kB activation. J Immunol. 2016; 196(1):132.134.

41. Albertsen HM, Chettier R, Farrington P, Ward K. Genome-wide association study link novel loci to endometriosis. PLoS One. 2013; 8(3):e58257.

42. Zhou Q, Jackson T, Elhakdakhny S, Townsend P, Crosbie E, Sayan B. Targeting the E3-ubiquitin ligase RNF144B to inhibit proliferation in

oestrogen receptor negative endometrial cancer cells. Eur J Cancer. 2016(61):S168-S169.

43. Wilkinson S, Bishop SC, Allen AR, McBride SH, Skuce RA, Bermingham M, Woolliams JA, Glass EJ. Host genetics of resistance to bovine tuberculosis infection in dairy cattle. In: Proc 67th Annual Meeting of the European Federation of Animal Science, Belfast, UK. 2016.

44. Ellis SA, Ballingall KT. Cattle MHC: evolution in action? Immunol Rev. 1999; 167(1):159-168.

45. Lewin HA, Russell GC, Glass EJ. Comparative organization and function of the major histocompatibility complex of domesticated cattle. Immunol Rev. 1999; 167(1):145-158.

46. Zare Y, Shook GE, Collins MT, Kirkpatrick BW. Genome-wide association analysis and genomic prediction of *Mycobacterium avium* subspecies paratuberculosis infection in US Jersey cattle. PLoS One. 2014; 9(2):e88380.

47. Riggio V, Pong-Wong R, Sallé G, Usai MG, Casu S, Moreno C, Matika O, Bishop SC. A joint analysis to identify loci underlying variation in nematode resistance in three European sheep populations. J Anim Breed Genet. 2014; 131(6):426-436.

2.3 Discussion (extension)

This Chapter set out to address the first objective of the present PhD thesis, namely to identify regions within the genome that are associated with susceptibility to bTB. Genomic association analyses identified new genomic regions associated with bTB. Analyses in this study involved a population that has not been studied in this manner before. Furthermore, a novel phenotype that has not been explored in previous studies was included in the analyses, where infection was determined by positive reaction to the skin test as well as positive post-mortem examination results even in the absence of a positive skin test; a further novelty in the definition was the introduction of a probability of infection in the original phenotype on which sire EBVs were based [15]. This is the phenotype currently considered in the official UK national genetic evaluation for bTB.

Sire de-regressed EBVs used in this study were based on disease incidents of respective daughters of the sires. Therefore, the de-regressed EBVs represent an aggregate of daughter contributions adjusted for environmental effects and effect of dams of daughters. This is more akin to the reliability of the breeding value hence it is likely to be more heritable. This could be the reason why high heritability estimates were observed in this study when de-regressed EBVs were used compared to the heritability estimate of 0.09 based on the probability of infection derived from the binary outcome [15]. Practically, de-regressed EBVs are advantageous in that data from un-genotyped individuals (e.g. daughter information in our case) can be used to generate phenotypes for genotyped ones (sires in our case). Data from the latter can then be used in the single-step approach whereby pedigree and genomic information

from un-genotyped and genotyped individuals, respectively, can be consolidated to estimate breeding values.

In the RHM analyses the region specific additive effect was estimated from the regional genomic relationship matrix (regional GRM) constructed from the 100 adjacent SNPs whereas the whole genome additive effect was estimated from the genomic relationship matrix calculated from all SNPs across the genome (global GRM). Similarly, the chromosome specific additive effect was estimated from the genomic relationship matrix constructed from all SNPs in the particular chromosome while the whole genome additive effect was calculated from the global GRM.

The regions identified by GWAS were confirmed by RHM and chromosomal heritability. As in previous studies of other populations and trait definitions, results from these analyses indicate that susceptibility of cattle to bTB is a heritable and likely a polygenic trait. Therefore, results support the concept of genomic selection. The genomic heritability estimates in this study were higher than The high genomic heritability compared to heritabilities based on pedigree information indicate that genomic selection will most probably yield quicker response to selection for resistance to bTB. However, prior to embarking on routine selection for resistance to bTB, it is important to assess its impact on the disease dynamics (Chapter 3 of this thesis) and the genetic gain for other economically important traits (Chapter 4).

CHAPTER 3

Impact of genetic selection for increased resistance to bovine tuberculosis on the disease transmission and dynamics

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3.1 Introduction

In the previous Chapter, genomic regions underlying variation in host response to bovine tuberculosis (bTB) infection were identified. The overarching outcome from results in that Chapter is that bTB is likely a polygenic trait, which is consistent with previous studies [1-4]. Consequently, selection for improved resistance to bTB in cattle should consider approaches that target variation contributed from across the entire genome. Consistent with this finding, in the present Chapter a stochastic model that combines quantitative genetics and epidemiological dynamics of bTB is used to investigate the impact of selection for enhanced bTB resistance on disease dynamics and prevalence.

As described in Chapter 1, bTB is an infectious zoonotic disease of cattle caused by *Mycobacterium bovis* (*M. bovis*) and is endemic in many parts of the world. Notably, bTB continues to be a challenge in the United Kingdom (UK) and Republic of Ireland (ROI) despite national eradication programmes running for over five decades hence breeding for resistance being proposed as an alternative strategy.

In the UK, sire genetic evaluations for susceptibility to bTB have been available to the dairy cattle industry since 2016. This information enables the industry to selectively use sires based on their inherent capacity to produce resistant progeny [5], consistently with the long-term practice to genetically improve other economically important traits. However, before embarking on intense selection for enhanced

resistance (or reduced susceptibility) to bTB, it is paramount to understand the consequences of such a selection process [6], with regards to its impact on disease risk and prevalence.

Chapter 1 presented the typical epidemiological model that has been used to study bTB dynamics in cattle. This model assumes that disease progresses through the Susceptible, Exposed, Test-sensitive and Infectious states (*SETI* model; Figure 1.1). Variations of this model have been used [7-14] but none of them has accounted for genetic variation in host resistance or considered genetic selection as potential control option. In the present study, we propose an epidemiological model which incorporates genetic variation of disease resistance in the host and may accommodate genetic selection.

Disease progression in the *SETI* model, as described in Chapter 1, is such that infected animals in the test-sensitive state which react positively to the skin test during testing are removed before they become infectious. If this is the case, identification of infected animals through frequent comprehensive testing as carried out in the UK, and immediate removal of test-positive animals before they can infect others should substantially reduce bTB prevalence. However, considering the continued persistence and general increase in bTB incidence in the UK [15], other models of disease transmission dynamics may need to be explored.

In the present study we consider a *SEIT* model where an animal becomes infectious (I) before it can be detected by the skin test (T). This model implies that infected cattle may become infectious, before they can be diagnosed. The model follows the suggestion that all tuberculous cattle with lesions, particularly in the respiratory tract, can be considered as potential excretors of *M. bovis*, thus constituting

sources of infection for other cattle both within and between herds [16, 17]. In the UK, 30-40% of animals that react positively to the skin test present visible bTB lesions during post-mortem examination [18]. Furthermore, unlike human TB, the latency of bTB is poorly classified and elucidated [19].

The purpose of the present study was to determine the impact of genetic selection for enhanced bTB resistance on disease transmission dynamics and prevalence using the *SEIT* model.

3.2 Material and Methods

Effects of selection for increased resistance to bTB on the risk and severity of bTB breakdowns were investigated on a simulated genetically heterogeneous population. The proposed genetic epidemiological model was implemented to simulate bTB disease dynamics in closed herds under the current UK bTB testing regime, firstly in the absence of selection and secondly following selection for enhanced resistance (reduced susceptibility) over 20 generations.

3.2.1 Simulated populations

Discrete, non-overlapping generations of dairy cattle populations (N=20,000) were generated comprising of 50% males and 50% females. A founder generation was created, where sires and dams were randomly chosen and mated to create the base population. This base population was generated assuming a sire to offspring ratio of 1:50, thus being consistent with the national policy in reporting genetic evaluations for bTB in the UK (R. Mrode, personal communication, 2017). A large family of half-siblings was thus created, which was reflective of the actual dairy cattle population structure, where, with the extensive use of artificial insemination, sires tend to have

large progeny (daughter) groups. Given that genetic selection of the best sires is the key component in selective breeding programmes in dairy cattle, selection was carried out based on estimated breeding values of sires generated as outlined below. This is also consistent with the current industry practice to only consider sire genetic evaluation for bTB.

3.2.2 Incorporating genetic variation in host susceptibility

Simulation of genetic variation for animal susceptibility to bTB assumed a normal distribution in the log scale, since previous studies had suggested that disease traits are usually skewed [20-24] and a log transformation is usually considered to achieve data normality. Log-normal distribution also ensures that susceptibility is positive. In addition, susceptibility was assumed to be a polygenic trait consistent with the infinitesimal model assuming many loci each with a small additive effect on the trait [1, 25]. Considering that either pedigree or genomic based methods will never capture all the genetic variance (σ_a^2) associated with a trait, both the true genetic value of an individual (TBV) and the estimated breeding value (EBV) were simulated drawing from normal distributions $N\sim(0, \sigma_a^2)$ and $N\sim(0, r^2\sigma_a^2)$, respectively, where *r* was the accuracy of the estimate. Thus, in the founder population, TBVs and EBVs were simulated from a $MVN\sim(0, \boldsymbol{G})$, where **G** corresponded to the variance-covariance matrix. The covariance between TBVs and EBVs was derived as follows:

$$cov_{TBV,EBV} = r * \sqrt{\sigma_a^2} * \sqrt{\sigma_a^2 r^2}$$

An additional term, the prediction error (PE) for each animal was computed as the difference between the TBV and the EBV.

In further generations, the TBV of the offspring of two selected animals was equal to the average TBV of the parents plus a Mendelian sampling term reflecting the random sampling of parental alleles. This term followed a normal distribution $N\sim(0, 0.5(1-\bar{F})\sigma_a^2)$, where $\bar{F}$ corresponded to the average inbreeding coefficient of the parents. In a similar way, the TBV of the offspring were decomposed into EBV and PE, both being computed as the average of their respective parental values plus the corresponding Mendelian sampling (MS) terms; the latter followed normal distributions $N\sim(0,\ 0.5(1-\bar{F})\sigma_{EBV}^2)$ and $N\sim(0, 0.5(1-\bar{F})\sigma_{PE}^2)$, respectively. Therefore, simulated TBVs, EBVs and PEs were computed for each offspring as:

$$EBV_{offspring} = \overline{EBV}_{parents} + MS_{EBV}$$

$$PE_{offspring} = \overline{PE}_{parents} + MS_{PE}$$

$$TBV_{offspring} = EBV_{offspring} + PE_{offspring}$$

In all generations, environmental effects were generated from a normal distribution $N\sim(0, \sigma_e^2)$, where σ_e^2 corresponds to the environmental variance; the latter was kept constant through all generations. Finally, the individual phenotypic values for underlying susceptibility were computed as the sum of the TBV of the animal plus the corresponding environmental effect.

3.2.3 Distribution of animals into individual herds

Currently, genetic evaluations for bTB in the UK assess the susceptibility of sires based on disease incidence of their daughters as described in Banos et al. [26]. Therefore, breakdowns were simulated here based only on female offspring produced in each generation. Female offspring were randomly allocated into 100 herds comprising 100 individuals each. Every sire contributed a minimum of two daughters

in at least two herds. Breakdowns were then simulated within each herd as outlined below.

3.2.4 The epidemic within herd transmission model

A stochastic within-herd bTB transmission model was developed to simulate bTB spread in each herd and to provide estimates of severity and duration of bTB breakdowns (Figure 3.1). In particular, a compartmental *SEIT* model was assumed in which susceptible cows may progress between the four infection states: 1) Susceptible state (*S*), where the animal is not infected but susceptible to infection, 2) Exposed state (*E*), where the animal is infected but not infectious and is undetectable by the skin test, 3) Infectious state (*I*), where the animal is able to infect others but is still undetectable by the skin test, 4) Test sensitive state (*T*), where the diseased animal is now detectable by the skin test. The model incorporates the current UK administration of a 60-day routine skin test performed on all animals in the simulated herds. At the specific test-days, infected animals at detectable state *T* may be diagnosed as reactors assuming a test sensitivity of Ω. Cows that react positively to the skin test are then culled immediately, in line with the UK official test-and-cull procedure (Figure 3.1).

Infection (transition from *S* to *E*) was modelled as a Poisson process with time dependent average infection rate $\lambda(t) = \alpha + \beta(I(t) + T(t))$, where $I(t)$ and $T(t)$ are the number of animals in the herd at the *I* and *T* states at time *t*, respectively and the parameters α and β represent transmission coefficients for external sources of infection (aggregate of all potential sources of external infection such as wildlife, infected move-in cattle and infected cattle from contiguous farms) and for within-herd cattle-to-cattle transmission, respectively (Figure 3.1) [12, 14]. We assumed a frequency dependent

mode of bTB transmission [27-29]. Progression of infected cows from *E* to *I* state and from *I* to *T* state occur at average rates σ and γ, respectively (Figure 3.1).

Individual variation in susceptibility was incorporated into the model through each individual's log-normally distributed susceptibility phenotype calculated as outlined above. The individual infection rate of individual j at time t was then defined as $\lambda_j(t) = e^{g_j}(\alpha + \beta(I(t) + T(t)))$, where g_j refers to the normally distributed susceptibility value specified in the genetic model above.

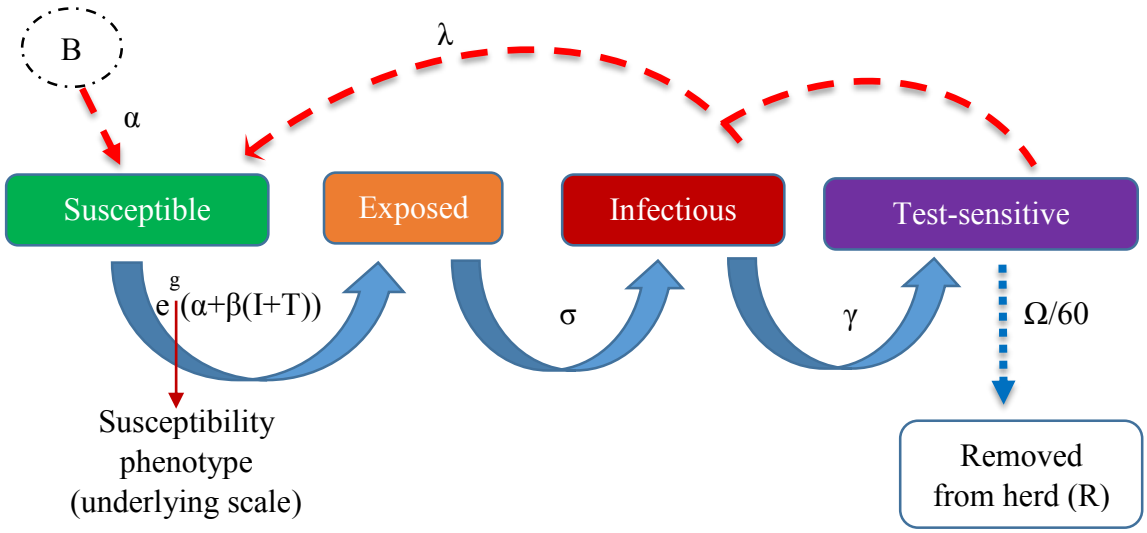

Figure 3.1 Different states of animal disease status in a *SEIT* model with genetic selection. Susceptible, Exposed (latent), Infectious and Test-sensitive (detectable) states are depicted. Once animals in the detectable state are diagnosed, they are removed from the herd (R). The background infection (B) and epidemiological parameters β, α, σ, γ and Ω are indicated. Genetic selection affects g (underlying susceptibility to bTB) determining the rate of progression from Susceptible to Exposed state and subsequently to other states.

To generate sufficient herds experiencing breakdowns in the first generation, the epidemic in each herd was started by two randomly chosen infectious individuals in state *I*, termed "index cases". Disease progression within each herd was then

simulated as a series of random independent events representing the transition of an animal between two successive states in the compartmental *SEIT* model. The time to the next event (inter-event time), the corresponding event type (for example, transition from *S* to *E*), and the corresponding individual experiencing the transition were determined using Gillespie's direct algorithm adapted to heterogeneous populations as outlined in [21]. In line with the current bTB control strategies, the epidemic in each herd was simulated until the end of a bTB breakdown defined by two consecutive negative skin tests for all herd members. During each epidemic the number of individuals in each disease state together with the corresponding times was recorded, and based on these, the total number of infected individuals and reactors, and the duration of each epidemic were calculated.

3.2.5 Model parameterisation

Input parameters for the epidemiological bTB model illustrated in Table 3.1 were based on real field data used for national genetic evaluations for bTB in the UK. This data consisted of 1,210,652 cow records from 10,589 herds where breakdowns had been declared between the years 2000 and 2014. The mean number of animals per herd in the dataset was 114, and the recorded number of infected animals referred to reactors diagnosed by the skin test. Based on the latest bTB epidemiological study in the UK [14] the value of the external force of infection α in the simulation (Figure 3.1) was set to $5x10^{-7}$ $days^{-1}$. Furthermore, a similar skin test sensitivity (Ω) of 0.60 as in Banos et al. [26] was used. To determine the remaining parameter values of the *SEIT* model (β, σ, γ, as well as genetic and environmental variance for underlying susceptibility and accuracy of selection), multiple parameter combinations were tested and the corresponding model output was compared to the following characteristics derived

from analysing the field data: mean percentage of skin-test reactors per breakdown (8.5%), mean duration of breakdown (366 days), and genetic variance (0.0032) and heritability (0.10) of the observed bTB phenotype indicating presence (reactor) or absence (non-reactor) of bTB.

The bTB susceptibility phenotype g in the *SEIT* model (Table 3.1) corresponds to the underlying scale of the binary presence or absence of the disease trait in the data analyses [26] (observed scale). In order to make the model results concordant with the observed scale, a range of different genetic and environmental variances and accuracy of selection for the underlying scale in the base population were explored and the corresponding heritabilities and genetic variances on the observed scale were calculated. The final genetic and environmental variances and accuracy of selection used to generate the base population were those that most closely mirrored the real field data estimates on the observed scale.

3.2.6 Selection process and impact

Firstly, the above-mentioned epidemic was allowed to run in simulation for 20 generations without any genetic selection in order to establish the baseline of bTB transmission dynamics. Subsequently, truncation selection of genetically resistant sires was simulated for 20 generations. Sires were selected based on their underlying susceptibility EBVs. Different levels of selection intensity were explored by selecting the 10, 25, 50 and 70% most resistant (least susceptible) sires. These reflect different potential selection strategies towards the disease. In the simulation, selected sires were randomly mated with cows. Dams were randomly selected in each generation. Population size and sex ratios were kept constant in each generation. The female offspring of these sires then formed the next generation of individuals for which bTB

epidemics were simulated. This way, selection impacted on the infection characteristic of daughters of the selected sires by increasing their genetic resistance (reducing susceptibility) to disease.

The impact of selection on bTB prevalence was assessed in each generation by the mean underlying susceptibility to bTB in the population as well as the average risk and severity of breakdowns. A breakdown was assumed to have occurred when there was at least one secondary case emanating from infection by the index cases within a herd. Therefore, the average risk of a breakdown (probability of a breakdown to occur) was calculated as the proportion of simulated epidemics per replicate that resulted in at least one secondary case (infected cow other than the index cases that seeded the epidemic), averaged over all replicates per generation. The severity of a breakdown was then assessed by the percentage of secondary cases within the breakdown and the induction time for secondary cases. The latter denotes the duration for production of secondary cases. Breakdowns were also categorised as mild, moderate and severe based on mean percentage of secondary cases being less than or equal to 3%, between 4% and 10% inclusive, and above 10%, respectively. Similarly, breakdowns were categorised as short, medium and long depending on whether the breakdown induction time for secondary cases was less than or equal to 180 days, between 181 days and 365 days inclusive, and above 365days, respectively.

Each selection scenario reflected one of the four selection intensities described above and was replicated 50 times. Results were averaged across all herds and replicates for each generation.

Finally, in order to assess the impact of the *SEIT* model assumption that animals become infectious first and then test-sensitive, the same simulations were run

separately assuming a *SETI* epidemiological model. In the latter, infected animals were test-sensitive, hence detectable, before they became infectious. The same parameters were used as for the *SEIT* model.

3.3 Results

3.3.1 Parameter values and model fit to real data

Parameter values were chosen so that simulated and real bTB breakdowns shared similar characteristics (Table 3.1, Figure 3.2). A strong similarity between real and simulated data was observed with regards to the distributions of mean percentage of reactors per breakdown, breakdown duration, and genetic and phenotypic variance and heritability of susceptibility on the observed scale (Table 3.1, Figure 3.2). The distributions of both the mean percentage of reactors per breakdown and the duration of breakdown were slightly more long-tailed in real data compared to simulated data, probably owing to the fact that extreme environmental conditions were not explicitly accounted for in the model. Significant correlations ($p<0.001$) were found between mean percentage of infected individuals per breakdown and mean duration of breakdown in both datasets; however, the correlation was smaller in real data (0.43) than in simulated data (0.85), for the same reasons as stated above.

The rate of progression from the E to I state, σ, corresponded to an exposed state duration ($1/\sigma$) of 25 days (Table 3.1). The rate of progression γ from I to T state suggested that, once an animal becomes infectious, it is expected to respond to the skin test within ($1/\gamma$) 2 days.

Table 3.1 Descriptive statistics of bovine tuberculosis epidemiological and genetic parameters in simulated and real data.

	Simulated data	Real data
Mean percentage of reactors to the skin-test (%)		
Average	8.7	8.5
Range (min-max)	0.0 - 70	0.08 – 98.0
3rd Quartile	10.0	9.5
Standard deviation	9.5	12.4
Mean duration of breakdown (days)		
Average	365.9	365.7
Range (min-max)	180.0 – 1,260	60.0 – 5,457
3rd Quartile	420.0	409.0
Standard deviation	174.7	395.1
Epidemiological parameters		
Rate of external infection (α) [days^{-1}]	5x10^{-7}	
Transmission coefficient (β) [days^{-1}]	0.012	
Rate from exposed to infectious state (σ) [days^{-1}]	0.04	
Rate from infectious to test-sensitive state (γ) [days^{-1}]	0.5	
Rate of detection (Ω)	0.60	
Genetic parameters of susceptibility		
Underlying scale		
Genetic variance	0.3	
Environmental variance	0.3	
Accuracy of selection	0.63	
Observed scale		
Genetic variance	0.0034	0.0032
Phenotypic variance	0.032	0.031
Heritability	0.106	0.103

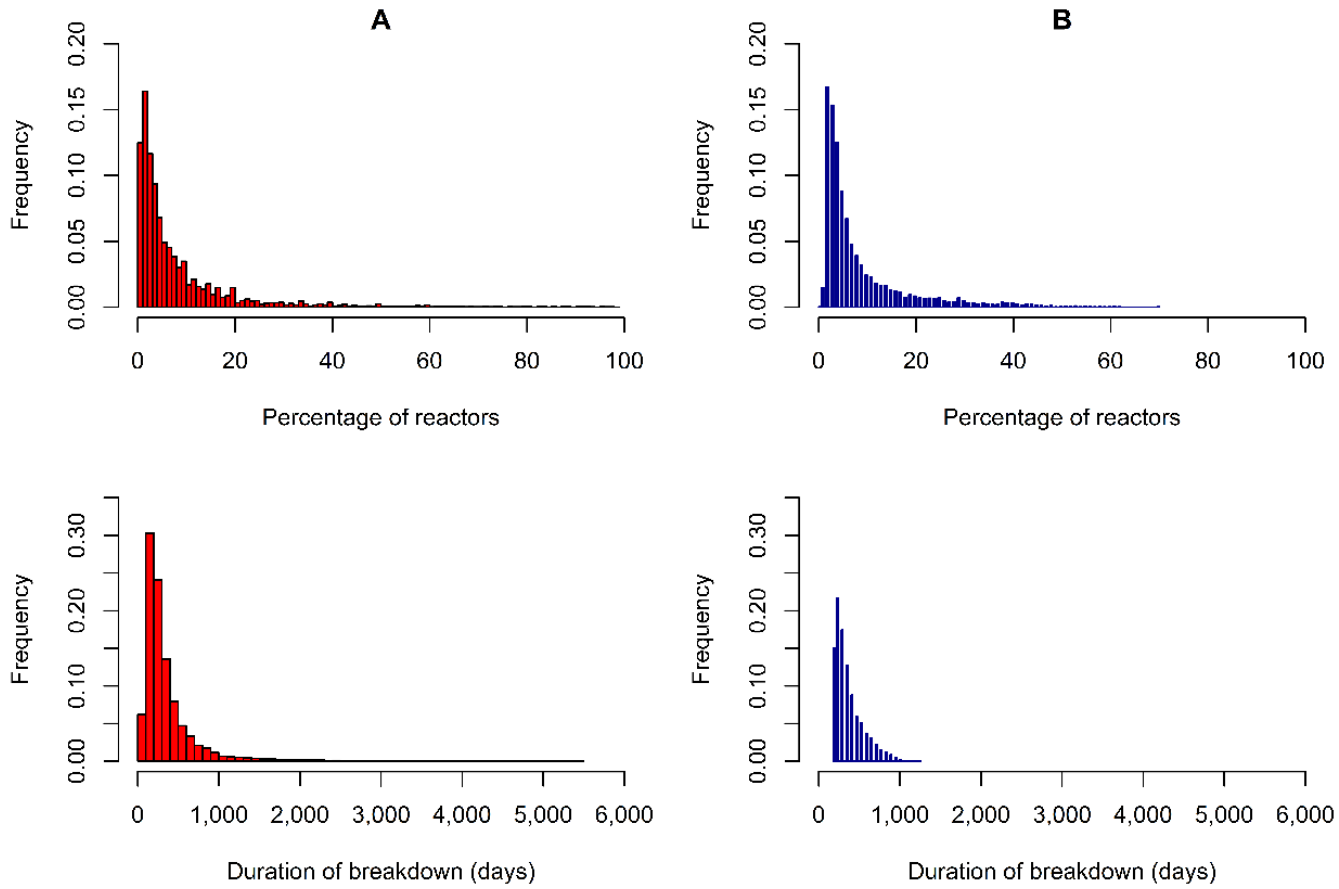

Figure 3.2 Distribution of percentage of reactors to the skin test per breakdown and duration of breakdown. Results from real data are given in red (A) and from simulated data in blue (B).

3.3.2 Impact of selection on underlying susceptibility scale

Genetic selection resulted in a decrease in the mean underlying susceptibility to bTB and the corresponding genetic variance (Figure 3.3). The initial underlying susceptibility phenotype in the base population was simulated with a mean of zero hence the decrease in susceptibility due to genetic selection is depicted by negative values in Figure 3.3. Greater reduction was observed for higher selection intensities. As expected, no change in genetic variance and mean susceptibility was observed across generations in the scenario of no selection.

3.3.3 Impact of selection on epidemic dynamics

Figure 3.4 shows the *SEIT* profiles (proportions of individuals in different states of the *SEIT* model over time) over successive generations for different selection intensities. The proportion of infected animals, including those in the exposed, infectious and test-sensitive states, was high before selection and significantly reduced after implementation of selection. There was no significant reduction in number of infected individuals and duration of the epidemic when no selection was performed across generations (Figure 3.4A). Numbers of susceptible and infected (*E*, *T* and *I* states) individuals and duration of the epidemic decreased with increased selection intensity (Figure 3.4B-E). For all selection scenarios, response to selection was fastest during the first five generations and slowed down in later generations.

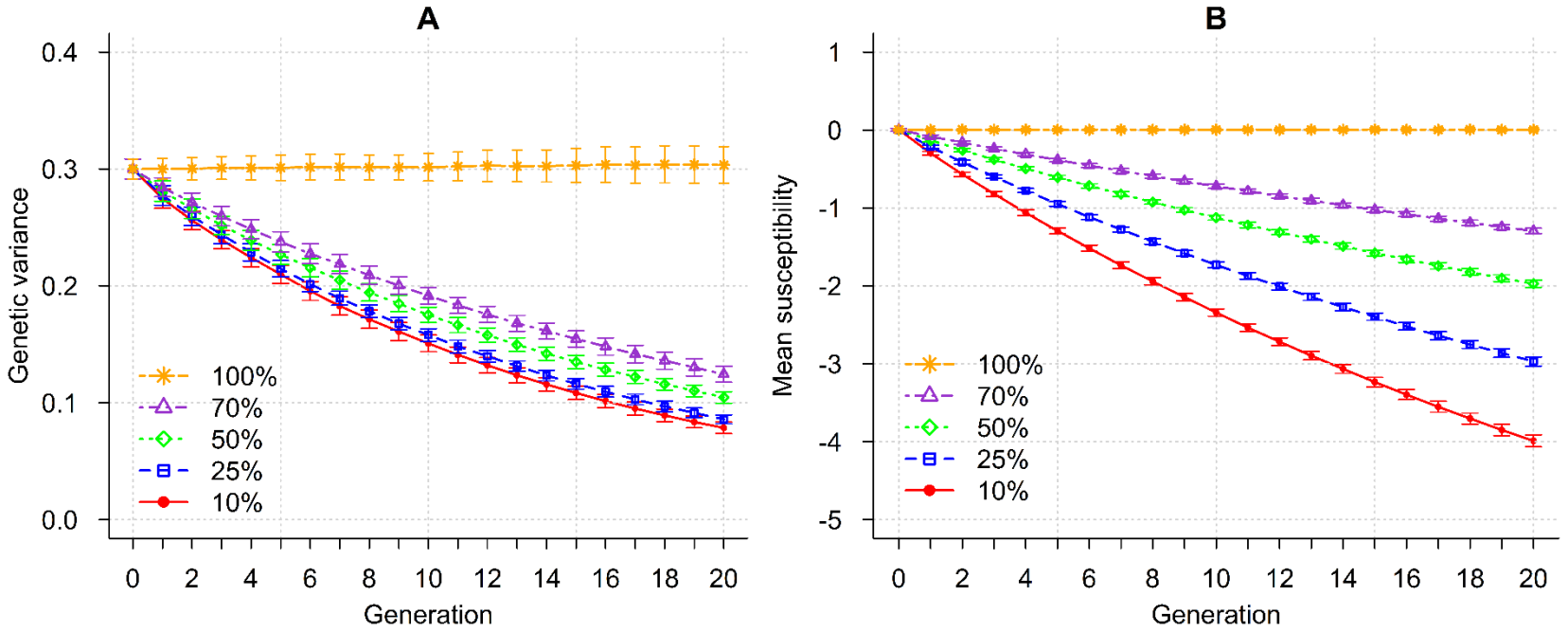

Figure 3.3 Impact of selection on underlying susceptibility to bovine tuberculosis. Changes in genetic variation (A) and mean susceptibility on the underlying scale (B); selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant of sires.

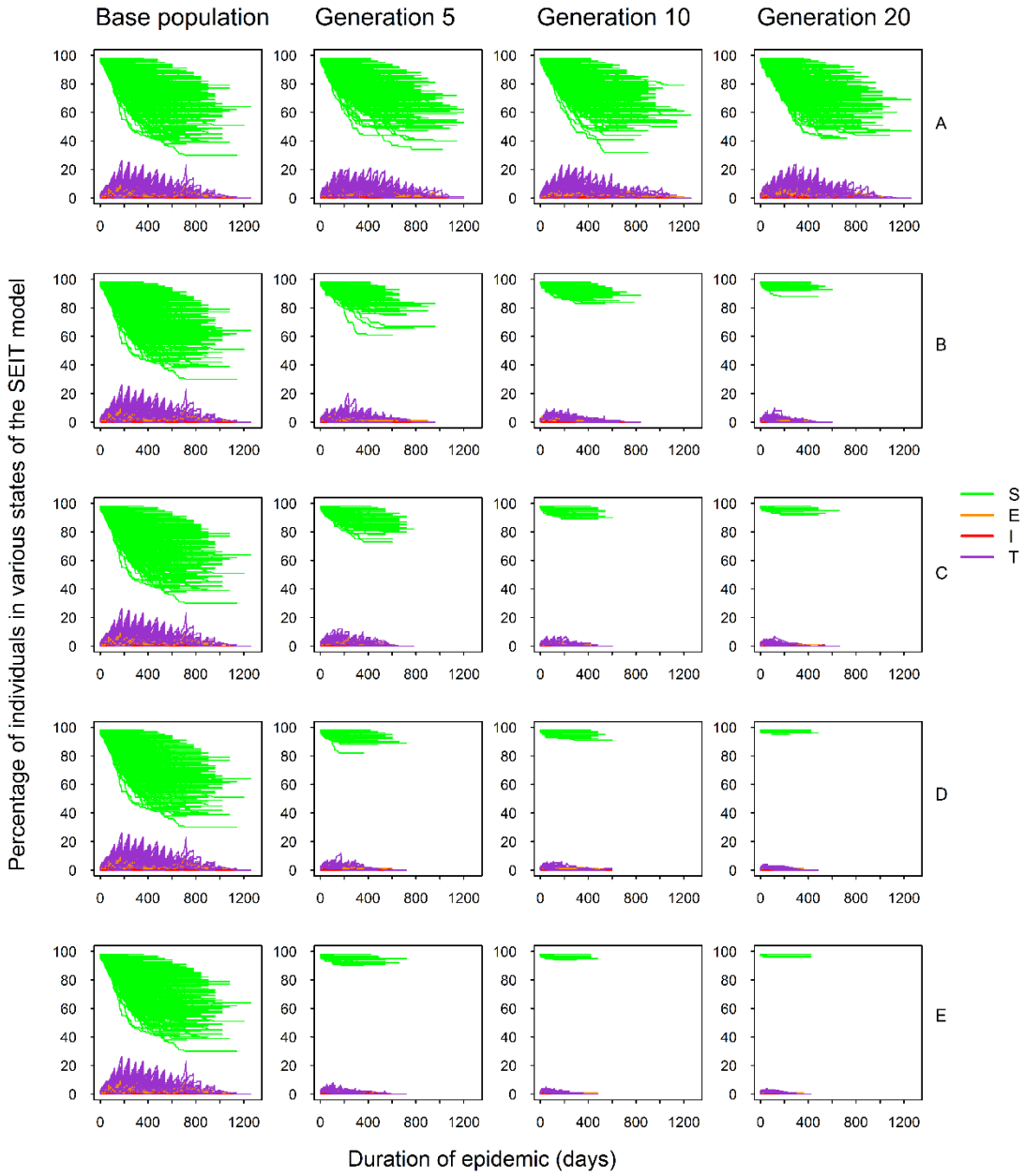

Figure 3.4 *SEIT* model profiles across generations for various selection intensities. Proportion of susceptible (*S*), exposed (*E*), infectious (*I*) and test-sensitive (*T*) individuals during the course of the epidemic; percentage of selected sires: 100% (no selection; A), 70% (B), 50% (C), 25% (D) and 10% (E).

3.3.4 Impact of selection on risk of breakdown

Figure 3.5 demonstrates a decrease in the probability of a breakdown to occur with increasing selection intensity. Prior to selection, the mean probability for occurrence

of a breakdown was 81.8%. When higher selection intensities were applied corresponding to selection of the 10 and 25% most resistant sires, this probability was halved after 4 and 6 generations, respectively. A similar result was achieved for lower selection intensities (50 and 70% most resistant sires) after 9 and 15 generations, respectively. Without genetic selection, the mean probability of a breakdown to occur stayed almost the same across generations (Figure 3.5).

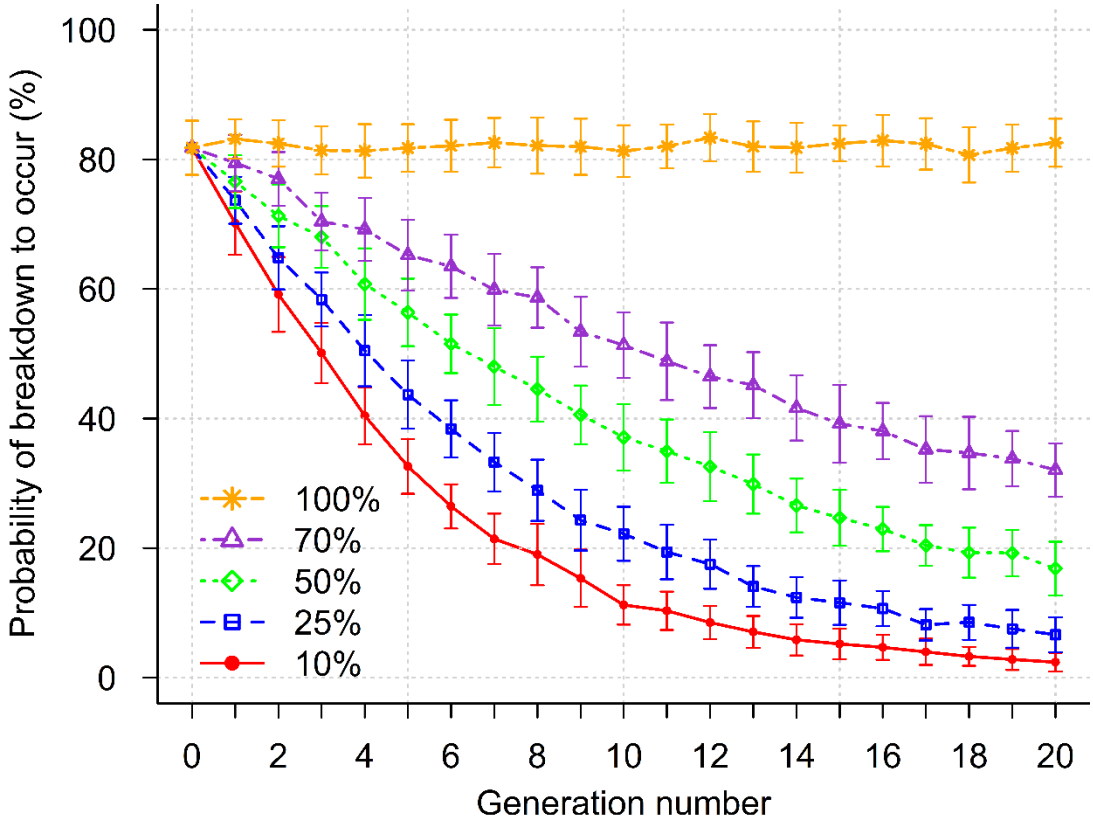

Figure 3.5 Impact of selection on risk of breakdown (probability of a breakdown to occur). Selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires.

3.3.5 Impact of selection on severity of the breakdowns

Selection led to a decline in the percentage of secondary cases per breakdown (Figure 3.6A). In the absence of selection the percentage of secondary cases and time for induction of secondary cases fluctuated around the initial mean (Figure 3.6). In order to reduce the mean percentage of secondary cases per breakdown to less than 1%, 4, 5, 7 and 11 generations of selection were required when 10, 25, 50 and 70% of the most resistant sires were selected, respectively. The corresponding average induction time for secondary cases in these generations reduced by more than half to 114.9, 125.5, 139.9 and 141.8 days for the four selection intensities, respectively, compared to 326.1 days before selection was introduced (Figure 3.6B). Furthermore, continuous selection for 12 and 17 generations was required to eliminate the epidemics (occurrence of secondary cases less than or equal to 0.1%) when the 10 and 25% most resistant sires were selected, respectively. However, elimination of bTB was not possible to achieve for lower selection intensities during the entire selection period of 20 generations.

The effects of genetic selection when breakdown severity was categorised are illustrated in Figures 3.7 and 3.8. Prior to selection, the proportion of mild, moderate and severe breakdowns was 0.46, 0.32 and 0.22, respectively. During selection, the proportion of mild breakdowns increased while the proportion of moderate and severe breakdowns decreased (Figure 3.7). When high selection intensities were applied (selection of the 10% or 25% most resistant sires), almost all breakdowns became mild by generation 10. However, it was only when selection of the 10% most resistant sires was implemented that almost all breakdowns became short at the end of selection (Figure 3.8). Proportion of long breakdowns was reduced by more than 50% after 1

and 2 generations for high (10 and 25% most resistant sires selected) and low (50 and 70% most resistant sires selected) selection intensities, respectively (Figure 3.8C). In the absence of selection, severity of breakdowns was almost the same, with slight fluctuations across generations.

Figures 3.5-3.8 collectively illustrate how genetic selection may decrease (i) the probability of a breakdown to occur and (ii) the severity of the breakdowns that do eventually occur.

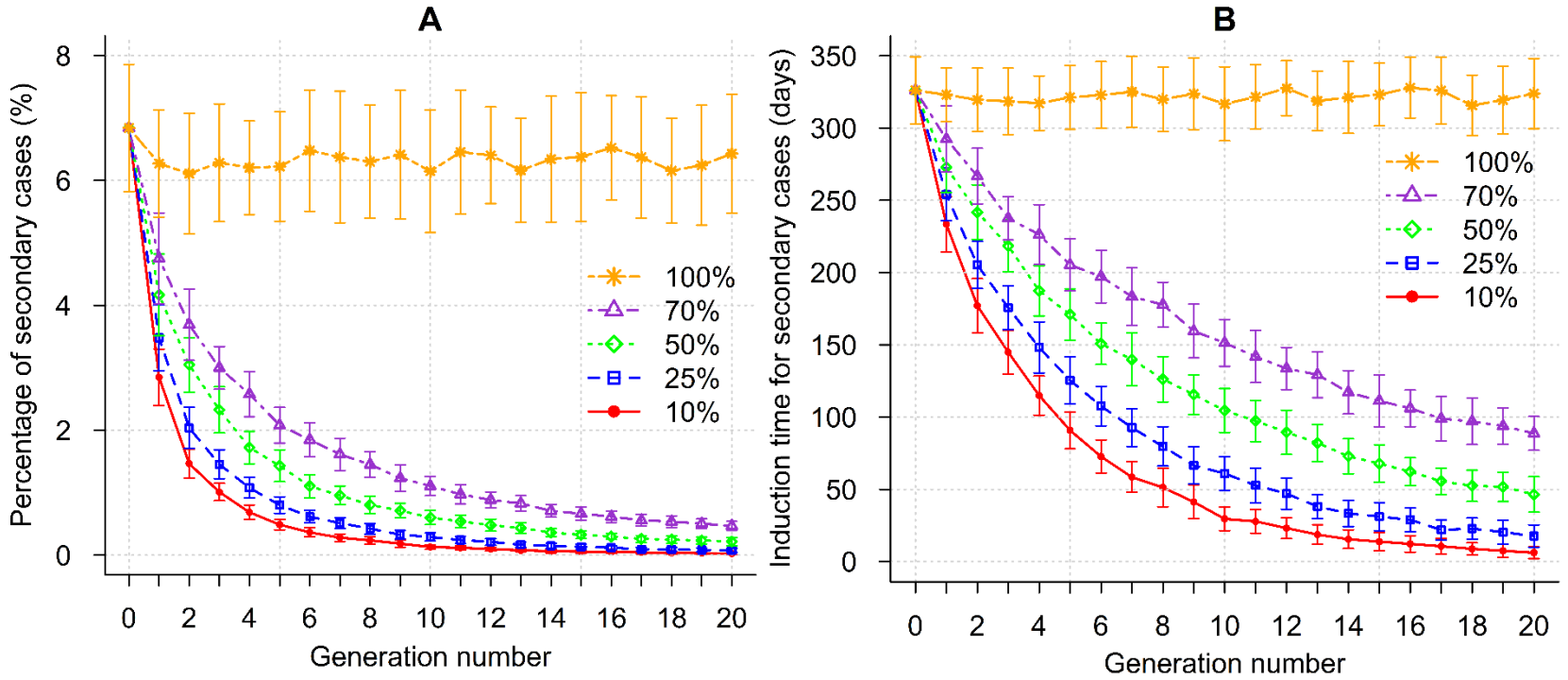

Figure 3.6 Impact of selection on mean percentage of secondary cases (A) and induction time for secondary cases (B) in breakdowns. Selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires.

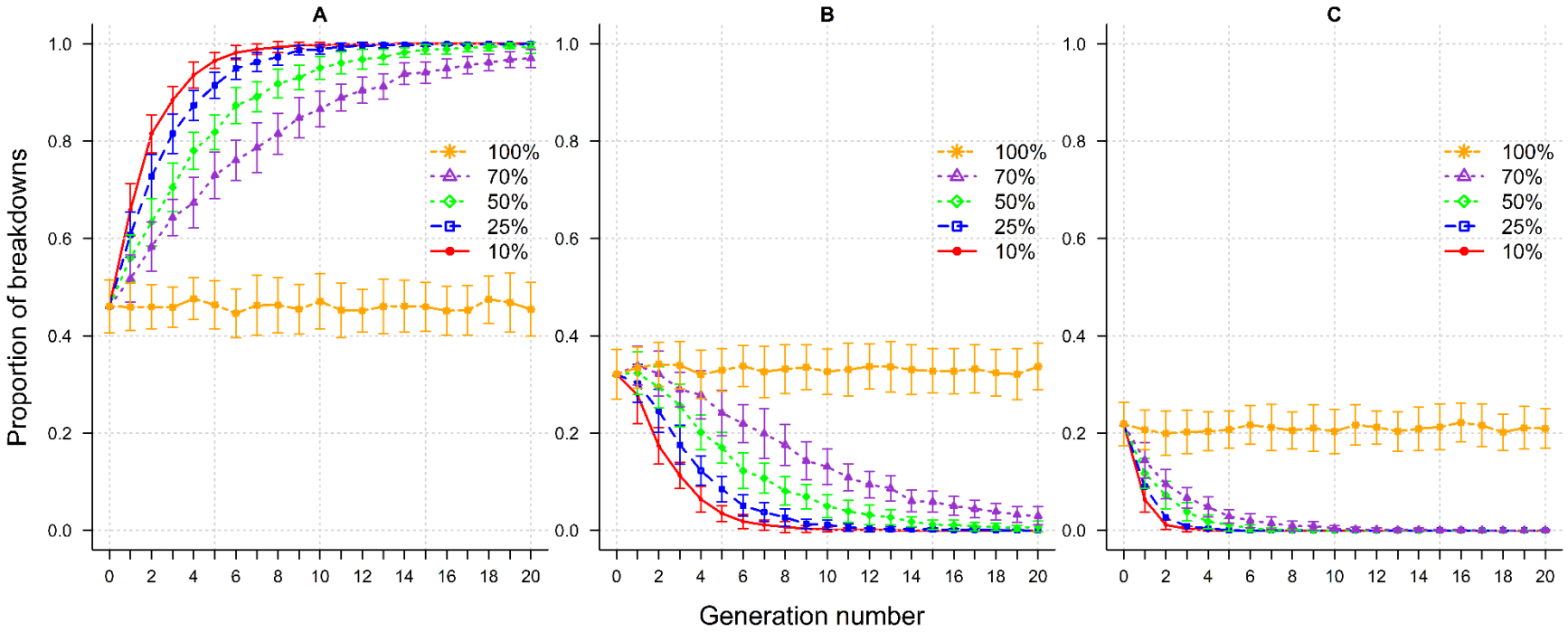

Figure 3.7 Impact of selection on severity of breakdowns (proportion of secondary cases). Breakdowns were categorised as mild (≤3% secondary cases - A); moderate (>3% but ≤ 10% secondary cases - B); and severe (>10% secondary cases - C); selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires.

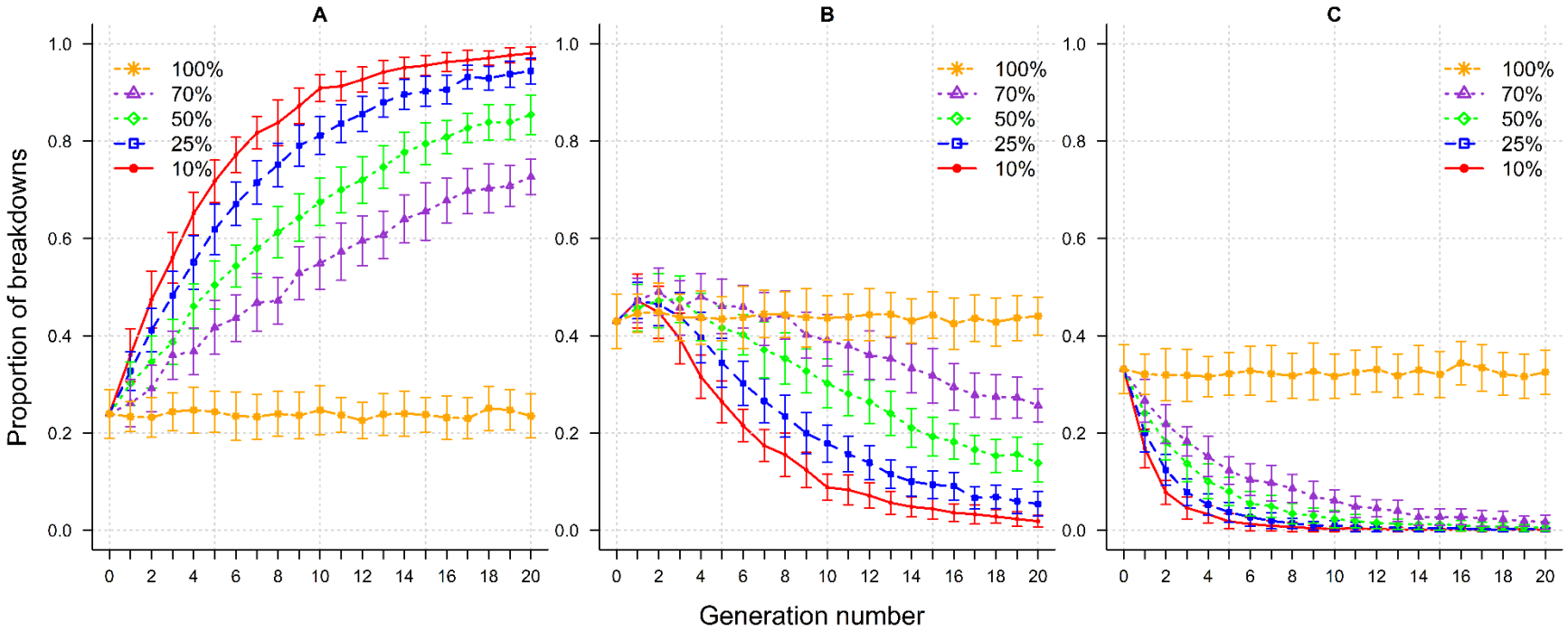

Figure 3.8 Impact of selection on induction time for secondary cases in breakdowns. Breakdowns were categorised as short (≤180 days - A); medium (>180 but ≤365 days - B); and long (>365 days - C); selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires.

3.3.6 Comparison between SEIT and SETI models

For the same parameter values, slightly more secondary cases per breakdown were generated with the *SEIT* (6.8%) compared to the *SETI* (5.8%) model in the base population (before selection). However, the impact of genetic selection on the average risk and severity of breakdowns under the two models were very similar (Figures 3.9, 3.10 and 3.11). For example, the same number of generations to reduce the probability of a breakdown to occur (risk) by half was needed in either model (Figure 3.9). Similarly, the difference in generations required to achieve a certain percentage of reduction (e.g. 50%) in secondary cases or time for induction of secondary cases between the two models was always less than one generation (Figures 3.10 and 3.11).

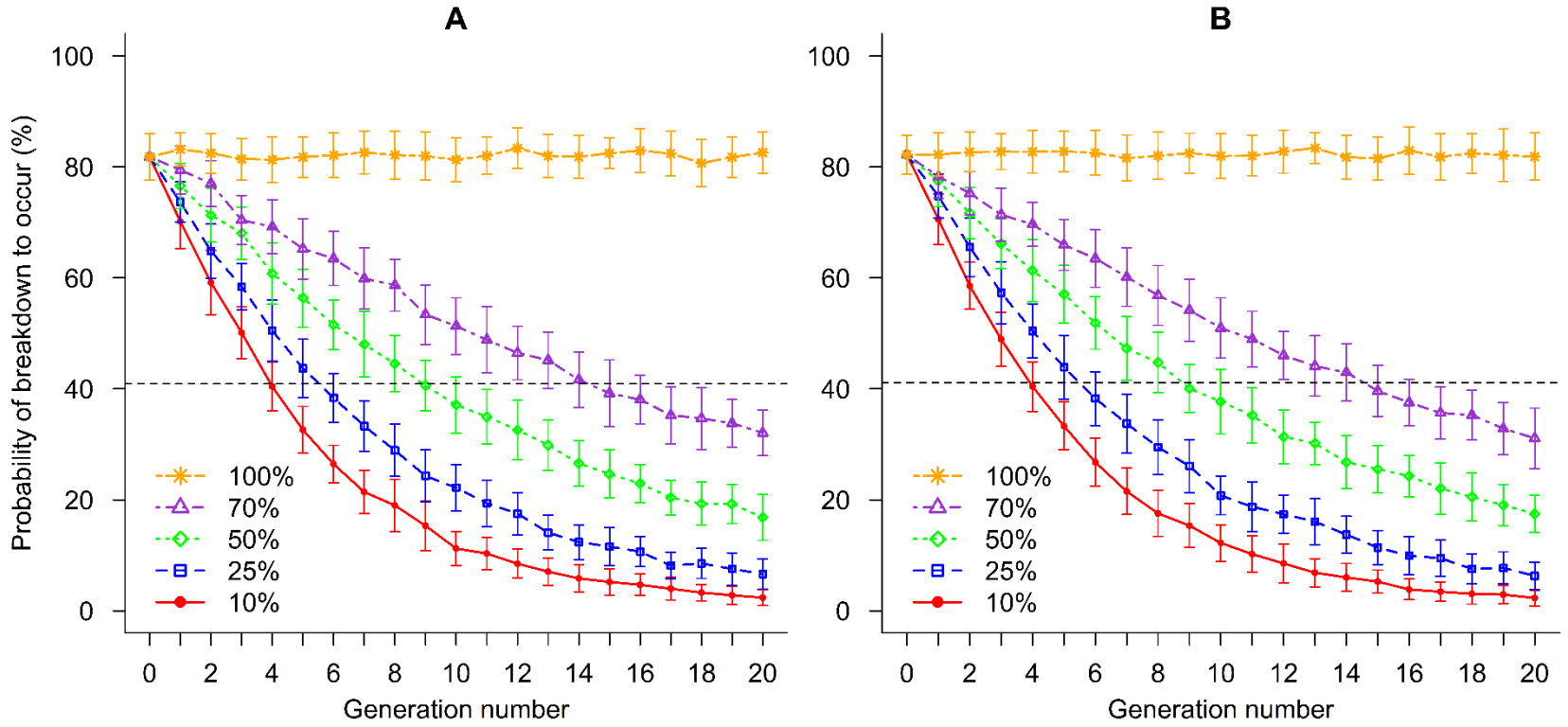

Figure 3.9 Impact of selection on average risk of breakdowns in the *SEIT* (A) and *SETI* (B) models. Selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires. The dashed horizontal lines represent reduction by 50%.

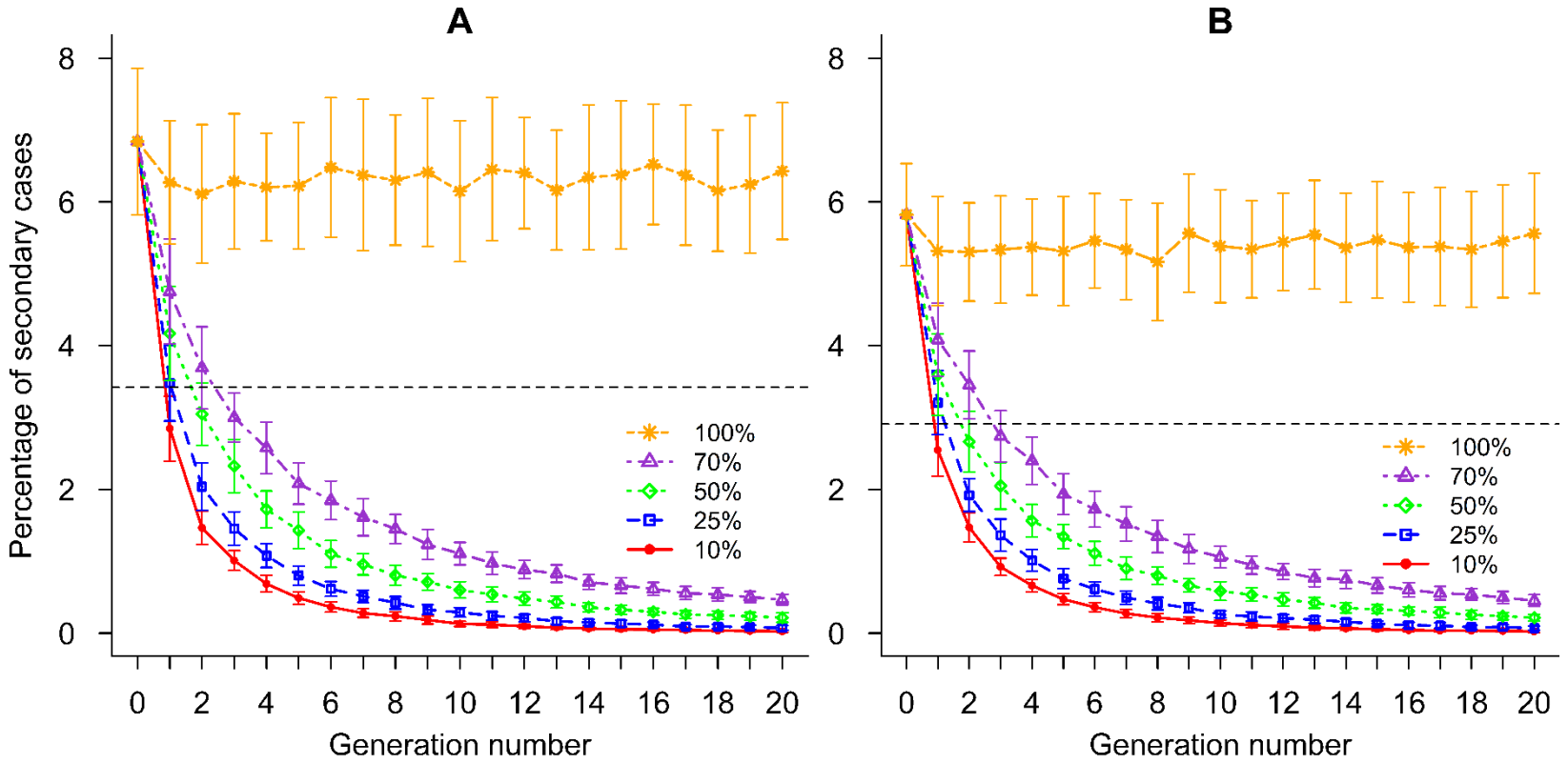

Figure 3.10 Impact of selection on severity of breakdowns (percentage of secondary cases) in the *SEIT* (A) and *SETI* (B) models. Selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires. The dashed horizontal lines represent reduction by 50%.

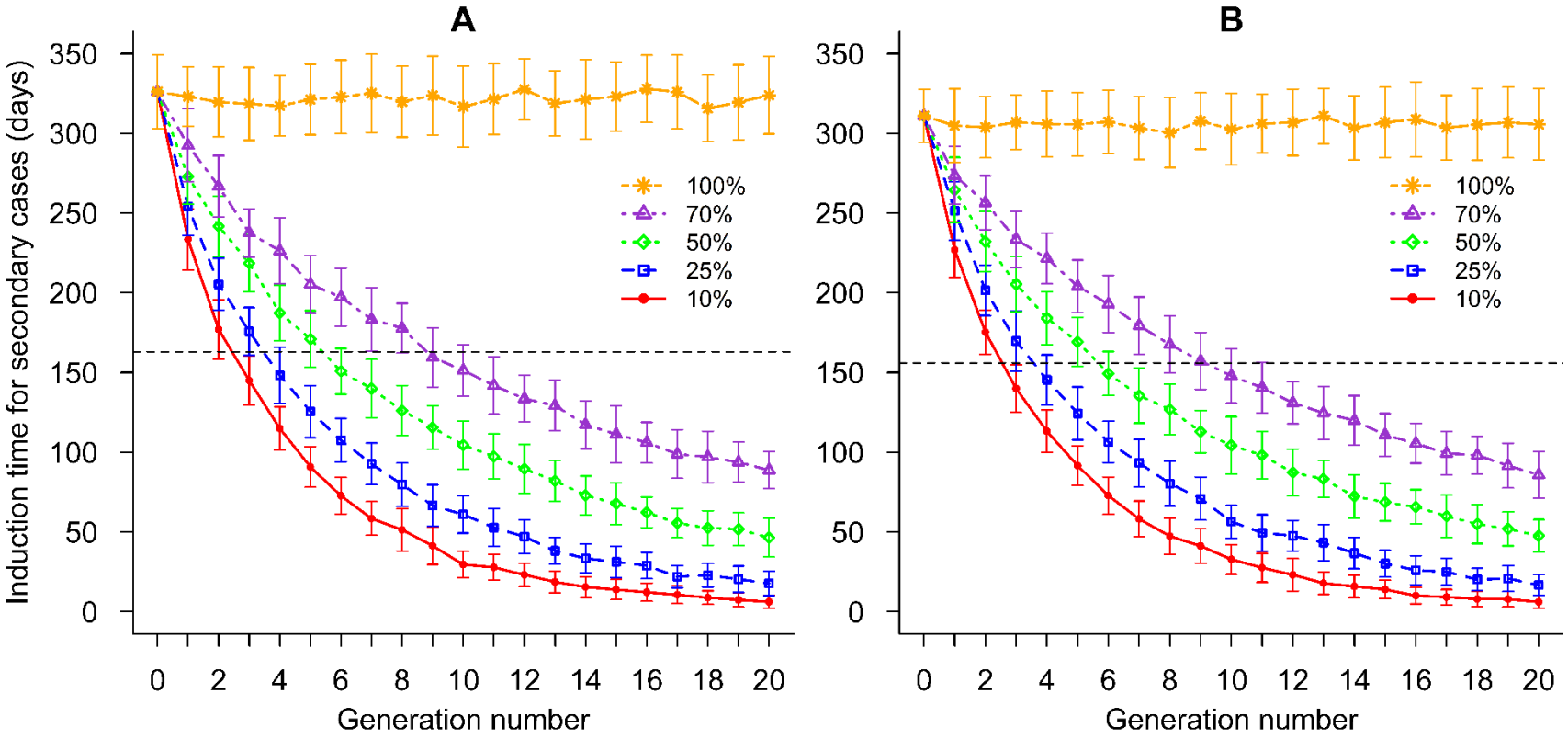

Figure 3.11 Impact of selection on severity of breakdowns (induction time for secondary cases) in the *SEIT* (A) and *SETI* (B) models. Selection intensities correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires. The dashed horizontal lines represent reduction by 50%.

3.4 Discussion

Considerable advances in infectious disease control may be achieved by selective breeding programmes that include disease resistance of animals in the breeding goal [30]. In this context, a breeding programme that exploits existing genetic variation in host susceptibility to bTB could form an important part of the national bTB eradication strategy [25, 26, 31-33]. However, quantitative genetics theory alone cannot predict how genetic gain in disease resistance translates into reduction of bTB breakdown risk and severity. The novelty of the present study lies on the development of a genetic epidemiological model that combines quantitative genetics and epidemiological dynamics of bTB, and can be used to quantify the consequences of genetic selection for enhanced resistance on disease prevalence and dynamics.

Parameterisation of the model using actual bTB data provided the opportunity to predict dynamics of the disease mimicking field conditions. Existence of a relationship between models and field or experimental data is essential for drawing reliable conclusions from data analyses [34]. Apart from slightly more skewed distributions in breakdown severity measures and lower correlations between percentage of reactors and duration of breakdowns in the real data, the developed model output was similar to results obtained from the field. In particular, the distributions of percentage of reactors to the skin test in both real and simulated data were characteristically skewed to the right and correlated with breakdown duration. The accuracy of selection from the present study was similar to that estimated for bTB (0.67) on the observed scale (real data). Additionally to between animal genetic variation [20], skewness in the distribution of disease traits may be attributed to environmental characteristics [35]. In the real data, factors such as differences in herd

size, management, badger prevalence and climatic conditions are likely to contribute to the diversity in epidemic characteristics [27, 36, 37].

Although the bTB model in the present study differs from previous epidemiological bTB models that did not incorporate genetic variation of the host, the estimated transmission coefficient β was within the range of transmission coefficients (0.006 to 0.014 $days^{-1}$) previously reported [8, 9, 13, 14, 38]. The length of exposed state (*E* state) in our model was 25 days, thus slightly higher than the 20 days estimated by O'Hare et al. [14] in a study conducted in the UK based on the *SETI* model. In our study an animal that became infectious was expected to become detectable within 2 days. This short time interval may be sufficient for some infected animals to infect others prior to diagnosis and subsequent removal from the herd. This could partly explain the persistence of bTB in the UK despite the on-going regime of skin test administration and slaughtering of positive reactors. The 2 days between *I* and *T* states in the present study may be comparable to the 1.8 days estimated by Conlan et al. [13], where early infectiousness was assumed (considering animals in both *E* and *T* states to be infectious) in the model explored. In their model the *E* state was referred to as the occult state, to denote that, although infectious, animals did not exhibit disease symptoms. From these findings it can be inferred that, once animals are infectious with regards to bTB, it takes a relatively short time before they could be detected by the skin test.

There are several important implications that arise from our results in so far as interpretation of bTB transmission and evaluation of control strategies are concerned, particularly in presence of genetic selection for increased disease resistance. Although the potential of the latter as a complementary strategy to conventional disease control

has been recognised [39], its utility in terms of reducing disease risk, prevalence and severity has not been previously evaluated.

In the model developed in the present study, susceptibility at the underlying scale reflected the probability of an individual to become infected. Therefore, as animals become more resistant, the expectation is for them to become less likely to be infected. Our results show that selection for resistance to bTB can indeed lead to a substantial reduction in the probability of experiencing a breakdown. Equally important, even when a breakdown was to occur eventually, it would more likely be less severe in terms of number of infected individuals and duration compared to a no selection scenario. Thus, our results are in agreement with previous studies that found that selection reduces both the risk and severity of epidemics for other diseases of livestock and fish [6, 20, 40-42]. This is expected to lead to a strong reduction, not only in frequency of future epidemics but also in economic losses, as prolonged breakdowns usually consume substantial resources. Furthermore, as selection reduces the number of reactors during a bTB breakdown, it is also expected to reduce the risk of recurrence [43, 44]. The latter has been found to be relatively high in the UK, where 23% (38%) of breakdowns recur within 12 (24) months under the same testing regime [45].

Reduction of mean host susceptibility through genetic selection is likely to favourably impact on another epidemiological parameter, R_0, which also determines risk and severity of infectious diseases [46]. Results of this study demonstrated that genetic selection significantly reduces the number of secondary cases that emanate from the index cases (from 6.8 to less than 1 secondary case in the first 5 generations for high selection intensities and 11 generations for low selection intensities) and based

on the definition of R_0, which is the average number of secondary cases produced by an infectious individual during the course of its infectious period, in a susceptible population [47, 48], it can be inferred that selection significantly reduces the average R_0 of the population. While R_0 can be affected by environmental factors [49, 50], studies on disease resistance have shown that genetic selection may reduce R_0 [6, 40, 41]. R_0 for bTB has been estimated to be between 1.3 and 1.9 in high risk areas and 0.6 and 1.4 in low risk areas in the UK [14]. Another bTB study in the UK estimated R_0 to range between 1.02 to 1.11 [51].

The amount of genetic progress in bTB resistance when superior sires were selected at different levels of selection intensity was explored. Simulating different selection intensities provides insight into future options for breeders. Our model predicted that most selection benefits would occur within the first 5-10 generations independently of the selection intensity applied. The lowest selection intensity considered here, corresponding to selection of the 70% most resistant sires, reflects a conservative approach often taken by breeders regarding novel traits in the breeding programme. Our results suggest that at this level, genetic selection alone will not eradicate bTB by the time England and Wales are set to achieve OTF status (year 2038, about 4-5 generations from now under conventional selection or about 2 generations under genomic selection). Thus, it will be worthwhile to consider medium to high selection intensities to quicken the eradication process. Higher selection intensities on bTB resistance would benefit high risk geographic areas where the disease is prevalent and/or endemic, and highly resistant sires may be required. However, care must be taken when higher selection intensities for bTB are sought due to possible unfavourable genetic correlations of bTB with other economically important traits.

Genetic selection could also be implemented alongside other interventions. In the context of the model, continued efforts to significantly reduce infection from external sources (α) i.e. wildlife-to-cattle and cattle-to-cattle transmission will likely expedite the eradication process. Furthermore, improvement of sensitivity of major bTB diagnostic tools such as the skin test and abattoir inspection could translate to increased removal rate of infected cattle hence reduce the overall herd infectivity. In high bTB prevalent areas, this could be coupled with shorter routine testing intervals to further accelerate diagnosis of infected animals. Other options not represented in the model such as selecting for increased resistance in cow dams in addition to sires and genetic selection to reduce infectivity in addition to susceptibility [52] could also be explored. The recent introduction of bTB genomic evaluations to facilitate genomic selection will also add to existing efforts. Despite genomic selection currently being undertaken at a relatively smaller scale compared to conventional selection, it however, has the potential to expedite the genetic gain and shorten the generation interval considerably [53, 54].

In order to assess the impact of selection on bTB prevalence and dynamics, the *SEIT* transmission model was adopted while a more optimistic *SETI* model in terms of transmission has been previously used in the majority of epidemiological studies. The results from the present study have demonstrated that the *SEIT* model indeed represented the "worst" case scenario as it resulted in more secondary cases per breakdown than the *SETI* model, owing to the fact that animals become infectious and can infect before they are detected and removed in the *SEIT* model. However, despite the difference between the models in terms of bTB transmission, the present study showed that the impact of genetic selection tended not to differ much between the two

models. This may be partly attributed to the relatively short time interval of 2 days estimated between the *I* and *T* states. Similarly, based on the number of secondary cases produced by index cases in this study it can be inferred that R_0 is likely to be higher for *SEIT* than *SETI*. However, the impact of selection on R_0 across generations is likely to be similar when the difference between *I* and *T* states is short (i.e. 2 days). Differences between the model predictions may be more pronounced if this time interval becomes longer and the contribution of the external force of infection (α) is higher.

Some important assumptions in the study need to be discussed. Although the model aimed to mimic the overall population structure of UK dairy herds, demographic characteristics and cattle movements were not explicitly included in the study. Future modelling studies that include a more explicit description of all relevant risk factors associated with bTB prevalence may be warranted to provide more accurate predictions of selection response. In the model, the external source of infection (α) was kept constant across generations. However, genetic selection is expected to reduce external infection. As more animals become resistant, fewer become infected and infectious hence this should reduce cattle-to-cattle and between cattle and wildlife transmission over time. Therefore, keeping the external source of infection constant in the simulations depicts a somewhat conservative approach with regard to the impact of selection. This study was undertaken to mirror the current situation where pedigree based EBVs for sires are being generated based on disease outcome of their daughters hence resembling more of progeny testing. Therefore, the Mendelian sampling was simulated with the same accuracy as the parental means. The model also assumes constant accuracy of selection across generations, however, in

reality this is expected to decrease over time with a decline in bTB outbreaks. Although the impact of reduction in accuracy of selection is expected across generations the effect on selection will particularly be low in later generations since most of the impact of selection is greater during the first few generations and less in later generations.

The genetic-epidemiological model developed in the present study provides the first quantitative estimates of the impact of selection for increased resistance on future bTB prevalence. The prospects of assimilating bTB resistance into the national selection programme are convincing despite the relatively low heritability of the trait. For example, while heritability of clinical mastitis in dairy cattle is low and unfavourably correlated with production traits, mastitis is nonetheless part of the selection index in several countries [55, 56]. The advantage of genetic selection is that effects are both cumulative and permanent; thus genetic selection can sustainably contribute to the control and ultimate eradication of bTB, as demonstrated by results in the present study.

3.5 Conclusions

We developed a genetic epidemiological model through which the impact of genetic selection for enhanced bTB resistance on disease prevalence and dynamics was quantified. Results demonstrated that genetic selection can substantially reduce bTB prevalence and severity of breakdowns across selection generations. Our study also highlights the importance of considering selection as a complementary strategy to existing interventions, especially with a view of accelerating the control and ultimate eradication of bTB to achieve the national goal of OTF status by 2038 envisioned in England and Wales. Future work should consider additional genetic selection strategies such as selection for resistant dams and selection on individual infectivity.

Finally, demonstrating that selection is likely to be successful is just one step in the decision making process. Before implementation of a breeding programme to select for bTB resistance another issue to be resolved is the effect on traits that already exist in the current breeding goal. This is addressed in the next Chapter of this thesis.

3.6 References

1. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, et al. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity. 2014; 112(5):543-551.
2. Richardson IW, Berry DP, Wiencko HL, Higgins IM, More SJ, McClure J, Lynn DJ, Bradley DG. A genome-wide association study for genetic susceptibility to *Mycobacterium bovis* infection in dairy cattle identifies a susceptibility QTL on chromosome 23. Genet Sel Evol. 2016; 48(1):1.
3. Finlay EK, Berry DP, Wickham B, Gormley EP, Bradley DG. A genome wide association scan of bovine tuberculosis susceptibility in Holstein-Friesian dairy cattle. PLoS One. 2012; 7(2):e30545.
4. Wilkinson S, Bishop S, Allen A, McBride S, Skuce R, Bermingham M, Woolliams J, Glass E. Fine-mapping host genetic variation underlying outcomes to Mycobacterium bovis infection in dairy cows. BMC Genomics. 2017; 18(1):477.
5. Agriculture and Horticulture Development Board (AHDB). TB advantage. http://dairy.ahdb.org.uk/technical-information/breeding-genetics/tb-advantage/ (Accessed 21st August 2017).

6. MacKenzie K, Bishop SC. Utilizing stochastic genetic epidemiological models to quantify the impact of selection for resistance to infectious diseases in domestic livestock. J Anim Sci. 2001; 79(8):2057-2065.

7. Barlow N, Kean J, Hickling G, Livingstone P, Robson A. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Prev Vet Med. 1997; 32(1-2):57-75.

8. Fischer E, Van Roermund H, Hemerik L, Van Asseldonk M, De Jong M. Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev Vet Med. 2005; 67(4):283-301.

9. Bekara MEA, Courcoul A, Benet J-J, Durand B. Modeling tuberculosis dynamics, detection and control in cattle herds. PLoS One. 2014; 9(9):e108584.

10. Kao R, Roberts M, Ryan T. A model of bovine tuberculosis control in domesticated cattle herds. Proc R Soc Lond B Biol Sci. 1997; 264(1384):1069-1076.

11. Biek R, O'Hare A, Wright D, Mallon T, McCormick C, Orton RJ, et al. Whole genome sequencing reveals local transmission patterns of Mycobacterium bovis in sympatric cattle and badger populations. PLoS Pathog. 2012; 8(11):e1003008.

12. Brooks-Pollock E, Roberts GO, Keeling MJ. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature. 2014; 511(7508):228-231.

13. Conlan AJ, McKinley TJ, Karolemeas K, Pollock EB, Goodchild AV, Mitchell AP, Birch CP, Clifton-Hadley RS, Wood JL. Estimating the hidden burden of bovine tuberculosis in Great Britain. PLoS Comp Biol. 2012; 8(10):e1002730.

14. O'Hare A, Orton RJ, Bessell PR, Kao RR. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. Proc R Soc Lond B Biol Sci. 2014; 281(1783).

15. Lawes J, Harris K, Brouwer A, Broughan J, Smith N, Upton P. Bovine TB surveillance in Great Britain in 2014. Vet Rec. 2016; 178(13):310-315.

16. Neill S, Hanna J, O'brien J, McCracken R. Excretion of Mycobacterium bovis by experimentally infected cattle. Vet Rec. 1988; 123(13):340-343.

17. McIlroy S, Neill S, McCracken R. Pulmonary lesions and Mycobacterium bovis excretion from the respiratory tract of tuberculin reacting cattle. Vet Rec. 1986; 118(26):718-721.

18. Broughan J, Harris K, Brouwer A, Downs S, Goodchild A, Upton P, Smith N. Bovine TB infection status in cattle in Great Britain in 2013. Vet Rec. 2015; 176(13):326-330.

19. Jones GJ, Pirson C, Gideon HP, Wilkinson KA, Sherman DR, Wilkinson RJ, Hewinson RG, Vordermeier HM. Immune responses to the enduring hypoxic response antigen Rv0188 are preferentially detected in Mycobacterium bovis infected cattle with low pathology. PLoS One. 2011; 6(6):e21371.

20. Gharbi K, Matthews L, Bron J, Roberts R, Tinch A, Stear M. The control of sea lice in Atlantic salmon by selective breeding. J R Soc Interface. 2015; 12(110):20150574.

21. Lipschutz-Powell D, Woolliams JA, Bijma P, Doeschl-Wilson AB. Indirect genetic effects and the spread of infectious disease: are we capturing the full heritable variation underlying disease prevalence? PLoS One. 2012; 7(6):e39551.

22. Anacleto O, Garcia-Cortés LA, Lipschutz-Powell D, Woolliams JA, Doeschl-Wilson AB. A novel statistical model to estimate host genetic effects affecting disease transmission. Genetics. 2015; 201(3):871-884.

23. Lipschutz-Powell D, Woolliams JA, Doeschl-Wilson AB. A unifying theory for genetic epidemiological analysis of binary disease data. Genet Sel Evol. 2014; 46(1):1-12.

24. Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. Superspreading and the effect of individual variation on disease emergence. Nature. 2005; 438(7066):355-359.

25. Raphaka K, Matika O, Sánchez-Molano E, Mrode R, Coffey MP, Riggio V, et al. Genomic regions underlying susceptibility to bovine tuberculosis in Holstein-Friesian cattle. BMC Genet. 2017; 18(1):27.

26. Banos G, Winters M, Mrode R, Mitchell A, Bishop SC, Woolliams JA, Coffey MP. Genetic evaluation for bovine tuberculosis resistance in dairy cattle. J Dairy Sci. 2016; 100(2):1272-1281.

27. Humblet M-F, Boschiroli ML, Saegerman C. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet Res. 2009; 40(5):1-24.

28. Brooks-Pollock E, Keeling M. Herd size and bovine tuberculosis persistence in cattle farms in Great Britain. Prev Vet Med. 2009; 92(4):360-365.

29. Reilly L, Courtenay O. Husbandry practices, badger sett density and habitat composition as risk factors for transient and persistent bovine tuberculosis on UK cattle farms. Prev Vet Med. 2007; 80(2):129-142.

30. Berry DP, Bermingham ML, Good M, More SJ. Genetics of animal health and disease in cattle. Irish Vet J. 2011; 64(1):5.

31. Brotherstone S, White I, Coffey M, Downs S, Mitchell A, Clifton-Hadley R, More S, Good M, Woolliams J. Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*. J Dairy Sci. 2010; 93(3):1234-1242.

32. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Brotherstone S, Berry DP. Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. J Dairy Sci. 2009; 92(7):3447-3456.

33. Richardson IW, Bradley DG, Higgins IM, More SJ, McClure J, Berry DP. Variance components for susceptibility to *Mycobacterium bovis* infection in dairy and beef cattle. Genet Sel Evol. 2014; 46(1):1.

34. Brooks-Pollock E, de Jong M, Keeling MJ, Klinkenberg D, Wood JL. Eight challenges in modelling infectious livestock diseases. Epidemics. 2015; 10:1-5.

35. Stear M, Boag B, Cattadori I, Murphy L. Genetic variation in resistance to mixed, predominantly Teladorsagia circumcincta nematode infections of sheep: from heritabilities to gene identification. Parasite Immunol. 2009; 31(5):274-282.

36. Karolemeas K, McKinley T, Clifton-Hadley R, Goodchild A, Mitchell A, Johnston W, Conlan A, Donnelly C, Wood J. Predicting prolonged bovine

tuberculosis breakdowns in Great Britain as an aid to control. Prev Vet Med. 2010; 97(3):183-190.

37. Skuce RA, Allen AR, McDowell SW. Herd-level risk factors for bovine tuberculosis: a literature review. Vet Med Int. 2012; 2012:article id 621210.

38. Perez AM, Ward MP, Charmandarián A, Ritacco V. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. Prev Vet Med. 2002; 54(4):361-372.

39. Allen AR, Minozzi G, Glass EJ, Skuce RA, McDowell SWJ, Woolliams JA, Bishop SC. Bovine tuberculosis: the genetic basis of host susceptibility. Proc R Soc Lond B Biol Sci. 2010; 277(1695):2737-2745.

40. MacKenzie K, Bishop S. A discrete-time epidemiological model to quantify selection for disease resistance. Anim Sci. 1999; 69(3):543-551.

41. Lipschutz-Powell D, Woolliams JA, Bijma P, Pong-Wong R, Bermingham ML, Doeschl-Wilson AB. Bias, accuracy, and impact of indirect genetic effects in infectious diseases. Front Genet. 2012; 3.

42. Nieuwhof GJ, Conington J, Bishop SC. A genetic epidemiological model to describe resistance to an endemic bacterial disease in livestock: application to footrot in sheep. Genet Sel Evol. 2009; 41(1):19.

43. Olea-Popelka F, White P, Collins J, O'Keeffe J, Kelton D, Martin S. Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland. Prev Vet Med. 2004; 63(3):163-172.

44. Wolfe DM, Berke O, Kelton DF, White PW, More SJ, O'Keeffe J, Martin SW. From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds. Prev Vet Med. 2010; 94(3):170-177.

45. Karolemeas K, McKinley TJ, Clifton-Hadley RS, Goodchild AV, Mitchell A, Johnston WT, Conlan AJK, Donnelly CA, Wood JLN. Recurrence of bovine tuberculosis breakdowns in Great Britain: risk factors and prediction. Prev Vet Med. 2011; 102(1):22-29.

46. Anderson RM, May RM, Anderson B. Infectious diseases of humans: dynamics and control: Oxford University Press, Oxford, UK; 1992.

47. Heffernan J, Smith R, Wahl L. Perspectives on the basic reproductive ratio. J R Soc Interface. 2005; 2(4):281-293.

48. Diekmann O, Heesterbeek JAP, Metz JA. On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases in heterogeneous populations. J Math Biol. 1990; 28(4):365-382.

49. Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD. Climate warming and disease risks for terrestrial and marine biota. Science. 2002; 296(5576):2158-2162.

50. Woolhouse ME, Dye C, Etard J-F, Smith T, Charlwood J, Garnett G, et al. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc Natl Acad Sci USA. 1997; 94(1):338-342.

51. Cox D, Donnelly CA, Bourne FJ, Gettinby G, McInerney JP, Morrison WI, Woodroffe R. Simple model for tuberculosis in cattle and badgers. Proc Natl Acad Sci USA. 2005; 102(49):17588-17593.

52. Tsairidou S, Anacleto O, Raphaka K, Sanchez-Molano E, Banos G, Woolliams JA, Doeschl-Wilson AB. Enhancing genetic disease control by selecting for lower host infectivity In: 11th World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand, 11-16 February. 2018.

53. Hayes BJ, Bowman PJ, Chamberlain A, Goddard M. Invited review: Genomic selection in dairy cattle: progress and challenges. J Dairy Sci. 2009; 92(2):433-443.

54. Schaeffer L. Strategy for applying genome-wide selection in dairy cattle. J Anim Breed Genet. 2006; 123(4):218-223.

55. Philipsson J, Lindhé B. Experiences of including reproduction and health traits in Scandinavian dairy cattle breeding programmes. Livest Prod Sci. 2003; 83(2):99-112.

56. Bell MJ, Pryce J, Wilson P. A comparison of the economic value for enteric methane emissions with other biological traits associated with dairy cows. American Research Journal of Agriculture. 2016; 2:1-17.

CHAPTER 4

Consequences of genetic selection for increased resistance to bovine tuberculosis on other economically important traits in dairy cattle

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4.1 Introduction

The previous Chapter of this PhD thesis demonstrated that genetic selection for enhanced resistance to bovine tuberculosis (bTB) would substantially reduce the disease prevalence and severity in future generations. The benefit was quantified under different selection scenarios. The next question concerns the impact of genetic selection for increased resistance to bTB on other economically important traits in the current UK breeding goal.

Presence of host genetic variation with regards to susceptibility to bTB [1-7] offers the possibility to selectively breed cattle for increased disease resistance. Moreover, as highlighted in previous Chapters, if selection is applied alongside other control interventions it will expedite control and eradication of the disease.

In the UK, routine genetic evaluations of individual animals for economically important traits related to animals' production, health, fertility and longevity have been available for a long time [8]. These genetic evaluations are used by the dairy industry to genetically select the best animals according to the breeding goal. Breeding goals may differ across farmers and regions within the country depending on the locally prevailing conditions and priorities. Nevertheless, a national overall selection index has been proposed, reflecting the overall dairy breeding circumstances. This index combines genetic evaluation for individual traits and weights them according to the traits' economic values [2, 8-10]. The overall selection index under which these traits

are combined is known as the Profitable Lifetime Index (£PLI) and is a genetic index assigned to each individual animal as a single aggregate value of the traits included. In the case of sire selection, the £PLI value represents the additional profit a high £PLI bull is expected to return from each of his milking daughters over their lifetime compared to an average £PLI bull [8]. Currently the percentage weightings based on economic value and variances of each trait in the UK overall selection index has placed increased emphasis on fertility, health and lifespan [8]. In the most recent index, these traits contribute 20.3, 21.6 and 14.4 %, respectively, to the index, while production stands at 32.2% [8]. The remaining 11.5% comprise cow maintenance and calving ease. As stated by Pritchard et al. [11] production previously constituted 75% and 45% of the selection index in 2003 and 2007, respectively. Reduced emphasis on production while emphasising on these other traits was aimed at redressing the decline in fertility [9, 12] and incidences of health problems [13] that resulted from early selection for increased milk production. Several studies have reported unfavourable correlations between health and production traits [14-17]. These concerns have coincided with the increase in incidences of bTB in the UK over the last two decades [18].

As of 2016, genetic evaluations for bTB are routinely calculated in the UK [8]. The availability of sire genetic evaluations for susceptibility to bTB provides the dairy cattle industry with an opportunity to avoid highly susceptible sires when other sires with the same performance on other traits are available. The use of highly resistant (low susceptibility) sires is anticipated to reduce bTB incidence, especially in high risk areas. The long-term impact of this selection on other traits already in the breeding goal is unknown. The genetic relationship between bTB and other economically important traits would largely dictate the way bTB is incorporated in the breeding

programme and addressed in selection practices. A negative favourable genetic correlation between confirmed *M. bovis* infection and milk yield in the UK dairy population reported by Brotherstone et al. [3] indicates that selection for increased resistance may not necessarily antagonise milk yield. However, a study in the Republic of Ireland reported a significant unfavourable genetic correlation of susceptibility to bTB with milk fat yield [19]. The same study, also reported unfavourable genetic correlations of bTB susceptibility with milk somatic cell score and body condition score, and a favourable genetic correlation with survival [19]. In another study, correlations between sire estimated breeding values (EBVs) for bTB resistance and other traits in the UK national breeding goal were generally weak and mildly favourable, especially with lifespan and overall index (£PLI) [2].

The objective of the present study was to investigate how long-term selection for enhanced resistance to bTB may affect other economically important traits in dairy cattle.

4.2 Materials and methods

A stochastic model was developed and applied to a simulated population that mirrored the structure of a real-life dairy cattle population under selection. The model was used to simulate genetic selection for reduced susceptibility to bTB and improvement in traits included in the current UK breeding goal. Description of simulated traits and weights for including them in selection indices are provided in Table 4.1.

4.2.1 Population

The procedure to generate the population was as described in Chapter 3 with some minor modifications. A base population of 20,000 animals consisting of 50% males

and 50% females was generated. Sire and dam true breeding values (TBVs) for susceptibility to bTB and the other traits in Table 4.1 were simulated from a multivariate normal distribution, $MVN{\sim}(0, \boldsymbol{G})$, where **G** is the variance-covariance matrix for all traits. The variance-covariance matrix was built on variance and genetic correlation estimates (Table 4.2) obtained from the literature [2, 3, 19, 20] and the UK Agriculture and Horticulture Development Board (unpublished data). In all cases, the variance-covariance matrix was positive definite. For all traits, the offspring TBVs were calculated as $TBV_{offspring} = \overline{TBV}_{parents} + MS_{TBV}$ where MS_{TBV} is the Mendelian sampling term, normally distributed with a mean of zero and variance equal to half the genetic variance of the particular trait.

After simulating individual traits, animal TBVs for each trait were combined into two selection indices: one mimicking the UK overall selection index (£PLI) and the other the UK fertility index. Traits were weighed by respective economic weights published in Interbull [20] that are shown in Table 4.1. To avoid the cumbersome process of recalculation of economic weights in the selection index [21] due to removal of traits from it, composite traits of feet and legs and mammary were included in the simulation and assumed to have zero correlation with bTB. In each case, one half of the TBV for each trait, reflecting the transmitting ability of the animal, was multiplied by the corresponding weight. The cross-products were then summed up to build the corresponding index.

4.2.2 Simulation of selection

Truncation selection of sires was simulated across 10 generations. Sires were selected based on their TBVs and randomly mated to dams. Each sire had to have at least two offspring per generation.

Table 4.1 Description of simulated traits and weights for inclusion in selection indices.

Trait group	Trait (abbreviation)	Economic weights [20]		Trait description
		OI	FI	
Production	Milk yield (MY)	-0.027		Milk yield (kg) in a 305-day lactation
	Fat yield (FY)	0.08		Milk fat yield (kg) in a 305-day lactation
	Protein yield (PY)	1.71		Milk protein yield (kg) in a 305-day lactation
Body conformation	Feet and legs (FL)	1.13		Composite of linear type traits related to legs and feet measured on a scale from 1 to 9; high values are desirable.
	Mammary (MAM)	1.18		Composite of linear type traits related to the udder measured on a scale from 1 to 9; high values are desirable.
Health	Somatic cell count (SCC)	-0.19		Number of somatic cells per ml of milk; low values are desirable.
Fertility	Calving interval (CI)	-0.35	-0.31	Interval between two consecutive calvings (days); low values are desirable
	Non-return at 56 days (NR56)	2.16	1.56	Non-return to service rate after 56 days. 1 = return to service and 2 = successful service
Longevity	Lifespan (LS)	25.4		Lifespan score (scale reflects number of lactations) computed from number of lactations completed; censoring accounted for by prediction of future survival based on MAM, FL, fore udder attachment and SCC [11]; adjusted for milk production; high values are desirable.

OI = Overall Index; FI = Fertility index

In each generation, independent culling levels selection was performed, whereby selection was first aimed to reduce susceptibility to bTB and then to improve the other traits. Regarding the latter, three scenarios were examined with selection being practiced on the overall index, milk fat yield (FY) or milk protein yield (PY). The scenario of selection on the overall index represents the current breeding goal in the UK and therefore was the trait of primary interest. However, the other scenarios of FY and PY were secondary and performed to demonstrate the effects of selecting for some traits (in this case production traits) in the overall index. Different levels of selection intensities were explored regarding susceptibility to bTB, based on selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires. For each level of selection intensity against bTB susceptibility, two levels of selection intensity for overall index, FY or PY were applied: selecting the best 5 and 10% of the sires that were left after the first round of selection on bTB. This design mimics a strategy whereby the dairy industry would be expected to avoid using highly susceptible sires first before focussing on the other traits. In all cases, the impact of selection on bTB and traits and indices in Table 4.1 was assessed based on the corresponding average genetic merit per generation across all animals and 50 replicates of the simulation.

4.3 Results

4.3.1 Selection on bovine tuberculosis and overall index

Figures 4.1 and 4.2 show genetic trends for all traits and indices after selecting first against bTB susceptibility (five intensity levels) and then for enhanced overall index (Table 4.1). Selection intensity in the latter corresponded to selection of the best 5 and 10% of sires in Figures 4.1 and 4.2, respectively. For all traits, the average genetic merit in the base generation was simulated to be zero. Results can be compared to a no

Table 4.2 Genetic variances (diagonal) and genetic correlations (above diagonal) among traits studied.

	bTB	MY	FY	PY	FL	MAM	SCC	CI	NR56	LS
bTB	**0.0032**[#]	-0.48[π]	0.39[§]	-0.1[#]	0[ŧ]	0 [ŧ]	-0.34[§]	0[#]	0[#]	-0.62[§]
MY		**557,440***	0.61[†]	0.85[†]	-0.07[†]	0[†]	0.18[†]	0.47 [†]	-0.54[†]	0[†]
FY			**649.8***	0.69 [†]	-0.04[†]	0[†]	0.19[†]	0.46[†]	-0.38[†]	-0.13[†]
PY				**468***	-0.07[†]	0[†]	0.22[†]	0.45[†]	-0.55[†]	-0.14[†]
FL					**2.55***	0.21[†]	0.04[†]	-0.01[†]	-0.21[†]	0.65[†]
MAM						**4.96***	-0.21[†]	0.14[†]	-0.13[†]	0.26[†]
SCC							**959.9***	0.13[†]	-0.09[†]	-0.08[†]
CI								**80.1***	-0.45[†]	-0.51[†]
NR56									**0.004***	0.23[†]
LS										**0.335***

bTB = susceptibility to bovine tuberculosis; MY = milk yield; FY = milk fat yield; PY = milk protein yield; FL = Feet and legs; MAM = mammary; SCC = milk somatic cell count; CI = calving interval; NR56 = non-return at 56 days; LS = lifespan

*Interbull [20]; [#]Banos et al. [2] ; [§]Bermingham et al. [19]; [π]Brotherstone et al. [3]; [ŧ] Assumed

[†]UK Agriculture and Horticulture Development Board (unpublished data)

bTB selection scenario illustrated with the selection of all (100%) sires with regards to bTB susceptibility.

As expected the amount of genetic change in bTB susceptibility across generations was proportional to selection intensity. No significant change in susceptibility to bTB was observed in the absence of selection.

Selection for reduced bTB susceptibility had no effect on the overall index, which exhibited the same genetic trend across generations regardless of the selection intensity applied on bTB. The genetic progress of individual traits was indicative of their genetic correlation with bTB and the dynamics stemmed from the genetic correlations among the other traits (Table 4.2). The trend was also affected by the sign of the weight in the overall index.

Genetic trends for milk production traits (MY, FY and PY) were affected by selection on bTB to an extent dictated by the genetic correlations considered (Figure 4.1). The average genetic merit of animals for MY increased in all cases in response to selection on the overall index. The genetic gain of MY per generation without prior selection on bTB was 186.9 kg. For selection intensities on bTB corresponding to selection of the 10, 25, 50 and 70% most resistant sires, the genetic gain in MY per generation was 388.8, 337.3, 288.4 and 253.4 kg, respectively (Figure 4.1). These genetic gains are reflective of the strong favourable correlation between bTB and MY assumed in the present study. Compared to MY, benefits for PY were lower due to a weaker correlation assumed between bTB and PY. The genetic gain of the latter per generation was 10.7, 10.4, 10.1 and 9.8 kg for selection of the 10, 25, 50 and 70% most resistant sires, respectively, compared to 9.2 kg when no selection on bTB was practised. On the contrary, due to the unfavourable genetic correlation assumed,

selection for bTB compromised genetic gains for FY. Prior to selection on bTB, genetic gain in FY per generation was 7.4 kg and it was reduced to 5.8, 5.0, 3.6 and 2.3 kg for selection of the 70, 50, 25 and 10% most resistant sires, respectively (Figure 4.1).

For body conformation traits, selection for reduced susceptibility to bTB had no effect on FL and MAM because a zero genetic correlation was assumed between these traits and bTB (Table 4.2).

In the absence of selection on bTB, improvement of SCC was manifested by a $4.3x10^3$ cells/ml average decrease per generation because it was selected against (negatively weighted) in the overall index (Table 4.1). SCC continued to be improved when bTB susceptibility was selected against with an intensity corresponding to selection of the 50 and 70% most resistant sires. However, higher selection intensities on bTB corresponding to selection of the 10 and 25% most resistant sires curtailed SCC improvement leading to increases by $2.0x10^3$ and $4.0x10^2$ per generation, respectively, despite ongoing selection on the overall index (Figure 4.1). This was the effect of the negative (unfavourable) genetic correlation of bTB with SCC assumed in the present study.

LS increased in all cases, initially due to the positive weight that LS receives in the overall index (Table 4.1) and then due to the favourable (negative) genetic correlation with bTB (Table 4.2). Therefore, the number of lactations a cow was likely to survive on average was enhanced by 0.40, 0.36, 0.33 and 0.30 lactations per generation for selection of the 10, 25, 50 and 70% most resistant sires, respectively, compared to 0.27 lactations per generation for the no bTB selection scenario.

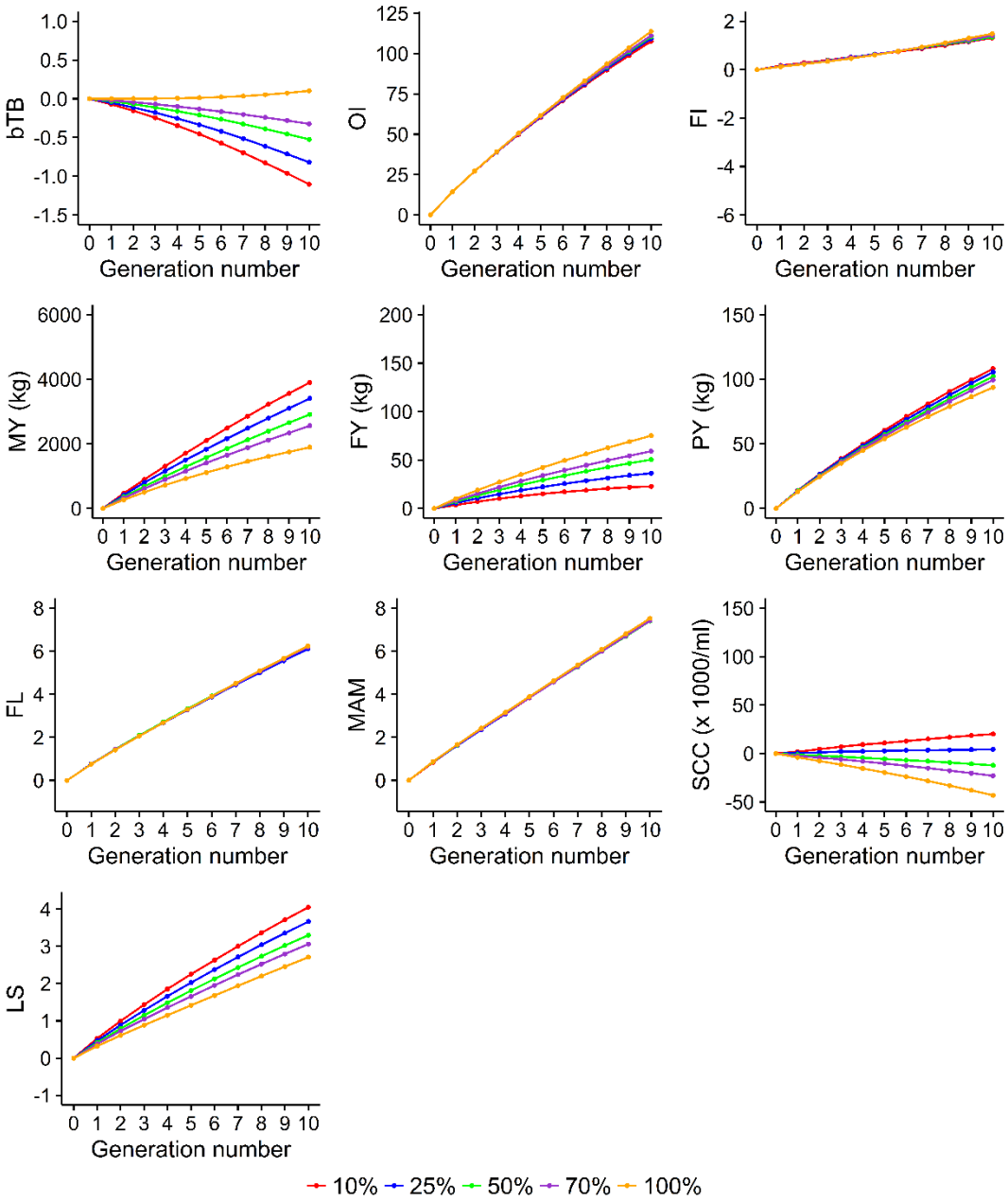

Figure 4.1 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 5% of remaining sires for increased OI. Vertical axes show the average genetic merit of all animals.

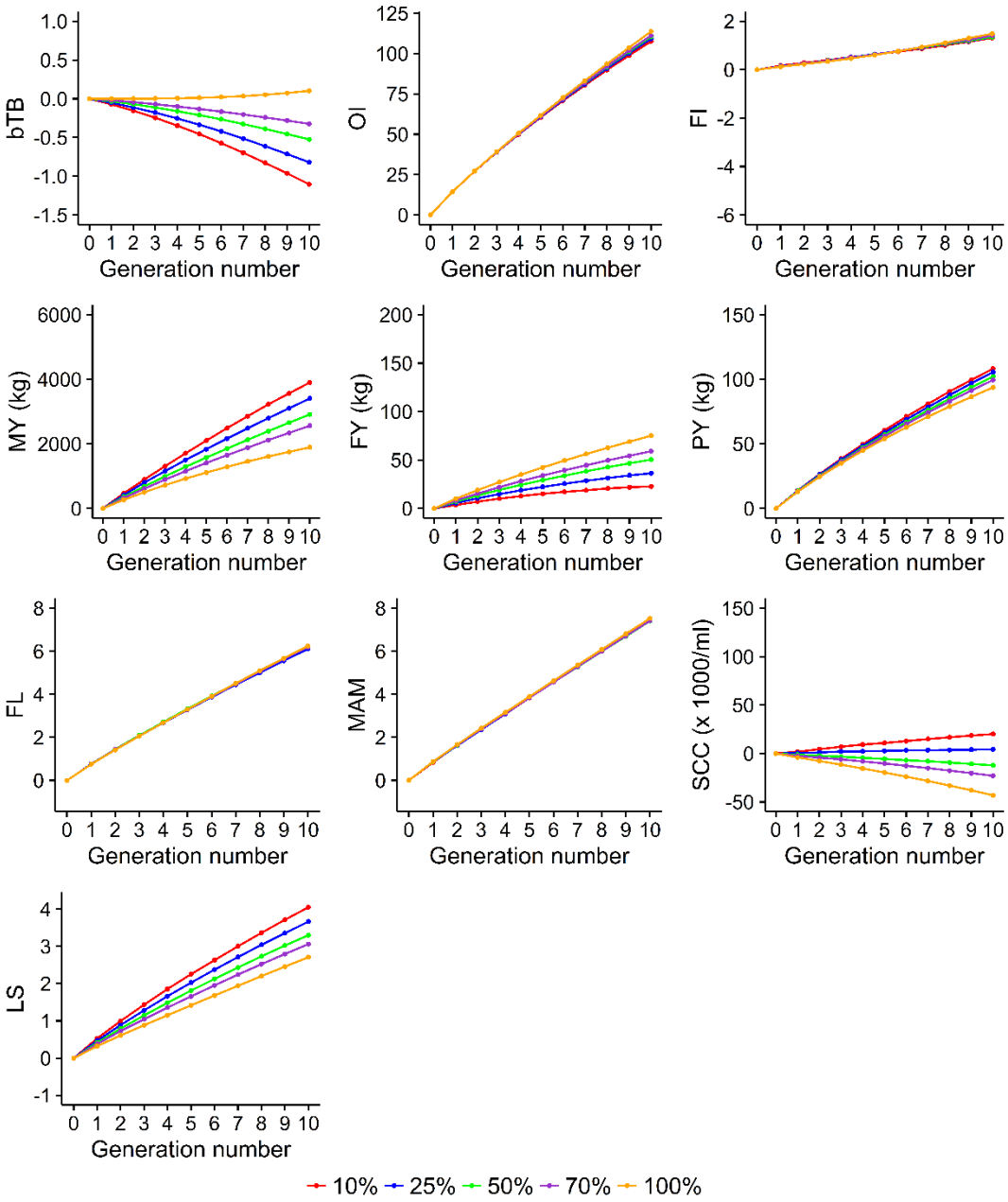

Figure 4.2 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 10% of remaining sires for increased OI. Vertical axes show the average genetic merit of all animals.

Regarding reproductive traits, since neither CI nor NR56 were assumed to be genetically correlated to susceptibility to bTB (Table 4.2), the same trend was expectedly observed for the fertility index independently of selection intensity on bTB.

A decrease in the intensity of selection for the overall index (Figure 4.2) reduced response to selection across all the individual traits and indices but the same comparative results regarding bTB selection remained.

4.3.2 Selection on bovine tuberculosis and fat yield

The genetic trends for all traits and indices after selecting first against susceptibility to bTB (five intensity levels) and then for improved FY are shown in Figures 4.3 and 4.4. The respective figures show results when the best 5 and 10% of sires were selected for FY. As before, all traits were simulated around a mean of zero in the base generation. For comparison with other selection intensities, a no bTB selection scenario corresponding to selection of all sires with regards to bTB susceptibility was included in the analysis.

Susceptibility to bTB increased in the scenario of no selection on bTB. This is explained by the antagonistic genetic correlation assumed between FY and bTB susceptibility. Therefore, increase in FY would result in a correlated increase in susceptibility to bTB even without selection on bTB. Otherwise, bTB susceptibility declined with intensified selection against it.

Due to high genetic correlation among production traits (Table 4.2), selection for increased FY resulted in a corresponding increase in MY and PY. As in the previous analysis, production traits were affected by selection against susceptibility to bTB because of the significant correlations considered in the present study (Figure 4.3). The average genetic gain in MY per generation was 356.4 kg for scenario of no

selection on bTB. After pre-selecting the 10, 25, 50 and 70% most resistant sires for bTB, MY genetic trend was 552.2, 507.6, 463.4 and 432.5 kg per generation, respectively (Figure 4.3), because of the relatively high and favourable genetic correlation between bTB and MY. The average increase in PY per generation as a result of selection on bTB was lower with values of 12.9, 12.7, 12.3 and 12.1 kg for selection of the 10, 25, 50, and 70% most resistant sires, respectively, compared to 11.3 kg when there was no selection for bTB. This was observed because bTB and PY were genetically weakly correlated. Contrastingly, genetic improvement for FY was hampered by selection on bTB. The scenario of no selection on bTB resulted in FY genetic gain of 16.0 kg per generation. However, genetic improvement for FY after selecting the best 10, 25, 50, and 70% bTB sires dropped to 11.6, 12.8, 13.9 and 14.6 kg per generation, respectively (Figure 4.3). This reflected the unfavourable genetic correlation between bTB and FY assumed in the current study.

In the present study, FY had a negative but weak genetic correlation with FL and had no correlation with MAM. Consequently, selection for increased FY caused a slight decrease in FL throughout the selection period while the trends for MAM stayed the same. Furthermore, due to the absence of genetic correlation between bTB and the latter traits assumed in this study, means for FL and MAM were similar across generations regardless of selection on bTB.

Given the unfavourable genetic correlation between FY and SCC assumed, trends of SCC increased with increase in FY, demonstrating a genetic deterioration for SCC and, consequently, mastitis resistance. These trends further increased with increasing selection intensity on bTB, reflecting a compounding effect of two sets of

unfavourable genetic correlations (FY with SCC and bTB with SCC) in the present study (Table 4.2).

In the absence of selection for bTB, LS declined by 0.07 lactations per generation as selection for increased FY was practiced, indicative of the unfavourable genetic correlation between the latter traits (Table 4.2). However, selecting for reduced bTB susceptibility, which was favourably correlated with LS, seemed to counter balance this effect. In fact, with increased selection intensity on bTB, follow-up selection of resistant bulls with high FY tended to improve LS. Selection of the 10, 25, 50 and 70% most resistant sires resulted in improvement of LS by 0.15, 0.10, 0.05 and 0.02 lactations per generation, respectively.

The selection criteria in the present analysis resulted in an increase in the overall index. However, selection against susceptibility to bTB did not affect genetic trends of the overall index, except a slight decrease in the index towards the end of the selection period for the scenario of no selection on bTB (Figure 4.3). This observation could be attributed to the dynamics emanating from the unfavourable genetic correlation between susceptibility to bTB and some of the traits in the overall index, particularly FY which is the trait that was directly selected upon. Selection for increased FY decreased the fertility index because of the negative genetic correlation between production and fertility traits (CI and NR56) assumed in the present study, which is widely reported in dairy cattle studies [9, 11, 22]. Fertility index trends did not vary according to selection applied to bTB, indicating the absence of genetic correlation between bTB and fertility traits studied (Table 4.2).

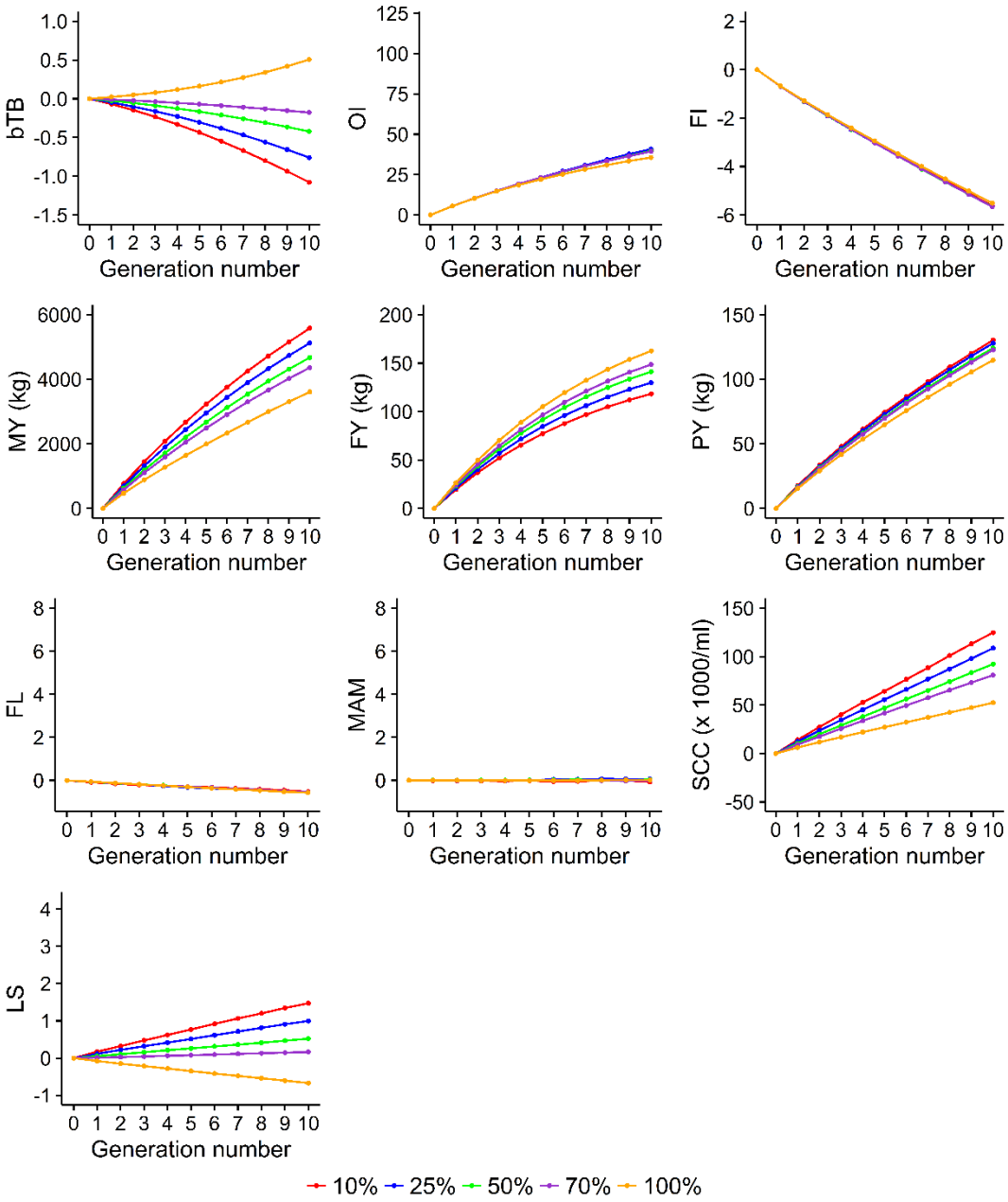

Figure 4.3 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 5% of remaining sires for increased FY. Vertical axes show the average genetic merit of all animals.

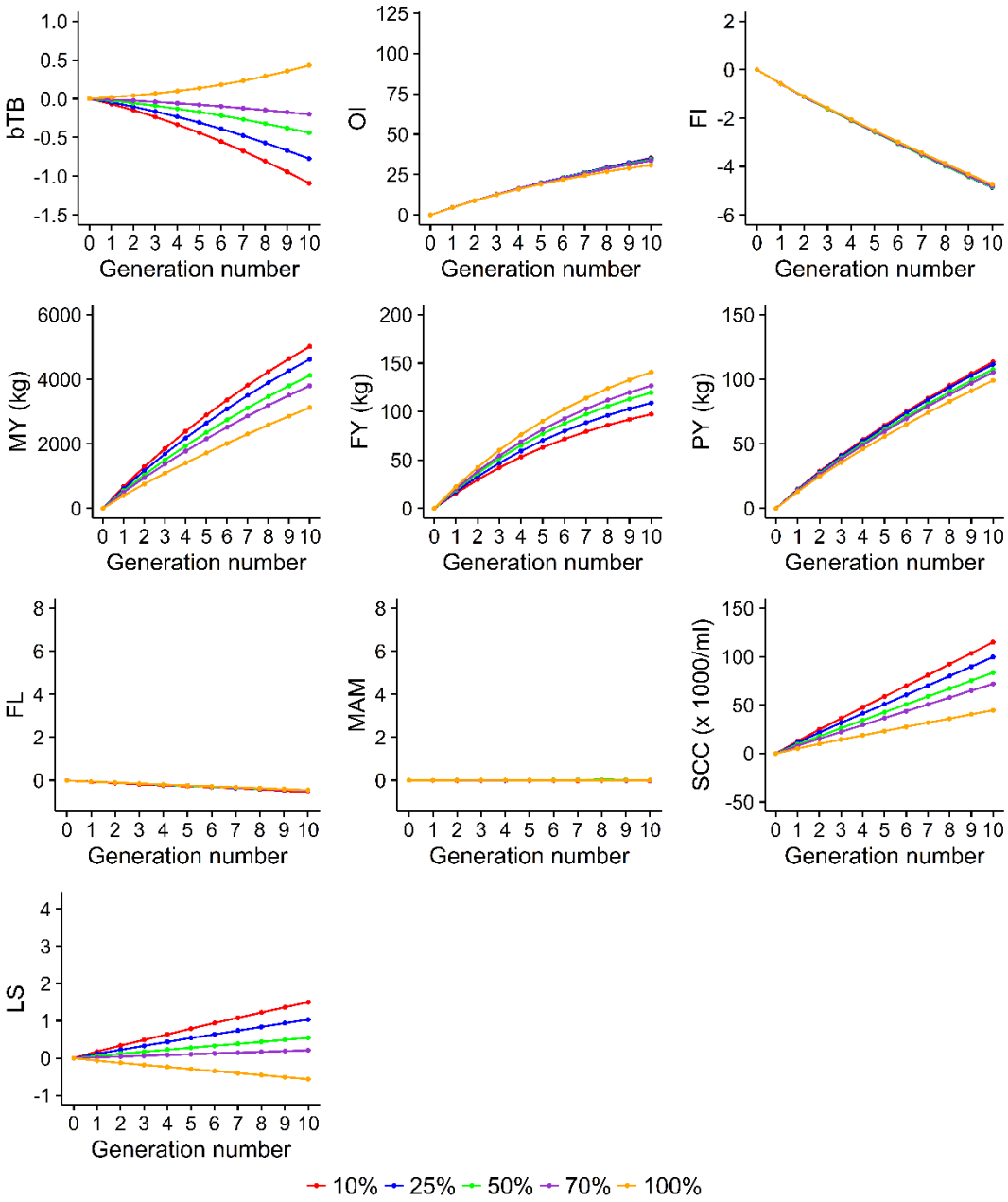

Figure 4.4 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 10% of remaining sires for increased FY. Vertical axes show the average genetic merit of all animals.

When selection intensity for FY was reduced, the response to selection in individual traits and indices also decreased but the variation of trends according to selection on bTB stayed the same (Figure 4.4).

4.3.3 Selection on bovine tuberculosis and protein yield

Figures 4.5 and 4.6 show genetic trends for all traits and indices after selecting for reduced susceptibility to bTB (five intensity levels) followed by selection for increased PY. The two Figures correspond to two selection intensities for PY (best 5 and 10% remaining after selection on bTB). Mean genetic merit in the base generation was generated around a mean of zero and trait means for selection on bTB were compared to the scenario of no selection.

When no selection was applied to bTB, susceptibility to bTB did not change significantly across generations, reflective of the weak correlation with PY. However, susceptibility to bTB declined with increasing intensity of selection against bTB.

Selection for increased PY also led to a proportional increase in other production traits owing to the high genetic correlation among them (Table 4.2). Trends for all the production traits varied according to selection for reduced bTB susceptibility mainly because of the genetic correlation of bTB with the former (Figure 4.5). However, while bTB was assumed to be favourably genetically correlated with MY and PY, it was unfavourably correlated with FY. Therefore, the average genetic gain in MY per generation was 569.1, 531.8, 499.7 and 476.9 kg for selection of the 10, 25, 50 and 70% most bTB resistant sires, respectively, compared to 443.5 kg per generation achieved in absence of selection on bTB. Genetic correlation between bTB and PY was weak hence selection of the 10, 25, 50 and 70% most resistant sires resulted in average protein production per generation of 14.2, 14.1, 13.9 and 13.8 kg,

respectively, compared to 13.6 kg when no selection for bTB was practised. However, the unfavourable correlation between bTB and FY led to a decline in FY as selection against bTB intensified. Prior to selection on bTB, FY increased by 13.4 kg per generation. However, improvement of FY was decreased to 8.6, 9.9, 11.1 and 12.0 kg when bTB selection was based on the 10, 25, 50 and 70% most resistant sires, respectively (Figure 4.5).

Selection for increased PY had a similar effect on FL and MAM as selection for increased FY. Because of the weak but negative genetic correlation of PY with FL and no correlation with MAM, a slight decrease in FL trends but no change in MAM trends were observed. Both FL and MAM did not vary with selection intensity on bTB because as assumed in the present study they were not correlated with the latter (Table 4.2).

Trends for SCC in this analysis were also similar to selection for increased FY. This was due to PY being positively (unfavourably) genetically correlated with SCC such that increasing PY resulted in an increase in SCC implying deterioration of mastitis resistance. This was exacerbated by the negative (unfavourable) genetic correlation between bTB and SCC. As a result, implementing selection of the 10, 25, 50 and 70% most resistant sires resulted in an increase of SCC per generation of $10.8x10^3$, $9.6x10^3$, $8.2x10^3$ and $7.3x10^3$ cells/ml, respectively, compared to $5.9x10^3$ cells/ml per generation when no selection on bTB was implemented (Figure 4.5).

In the present study, PY and LS were negatively (unfavourably) correlated meaning that increase in PY antagonised LS improvement. Therefore, in the absence of selection on bTB, LS declined across generations. In fact, LS declined at a rate of 0.072 and 0.026 lactations per generation when no selection and selection of the 70%

most resistant sires were applied, respectively. However, intensified selection against bTB susceptibility had a counteractive effect as it improved LS due to the favourable genetic correlation between the two traits. LS was enhanced as selection of the 10, 25 and 50% most resistant sires resulted in 0.086, 0.043 and 0.00058 lactations per generation, respectively.

Selection for reduced susceptibility, followed by selection for increased PY resulted in an increase in the overall index. However, the same selection criteria led to a decline in fertility index. The decrease in fertility index observed was most probably due to the negative unfavourable genetic correlation of bTB with fertility traits (CI and NR56). The two indices were nonetheless, not affected by selection on bTB.

Trends for individual traits and indices were lowered by reduction on intensity of selection for PY (Figure 4.6).

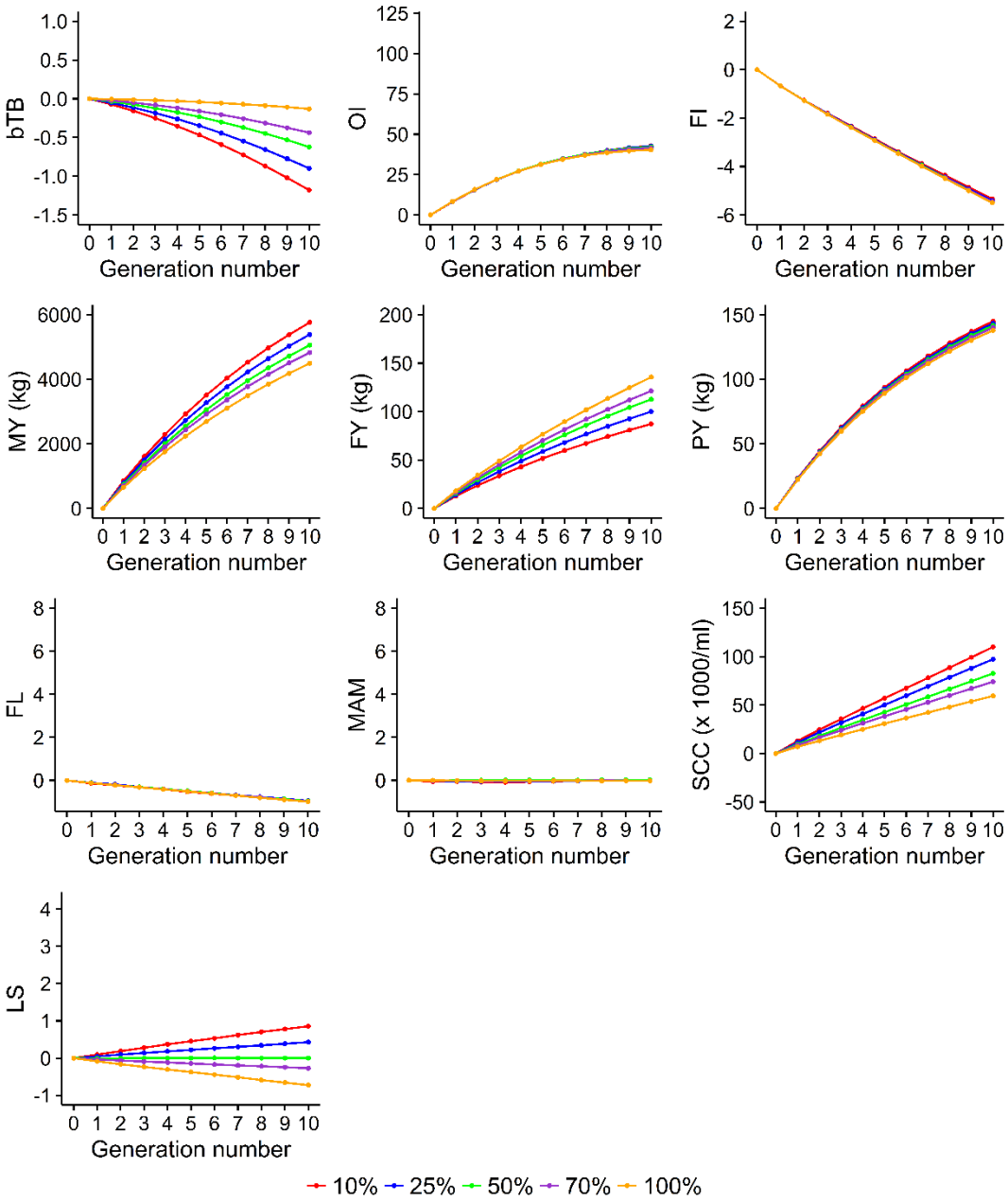

Figure 4.5 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 5% of remaining sires for increased PY. Vertical axes show the average genetic merit of all animals.

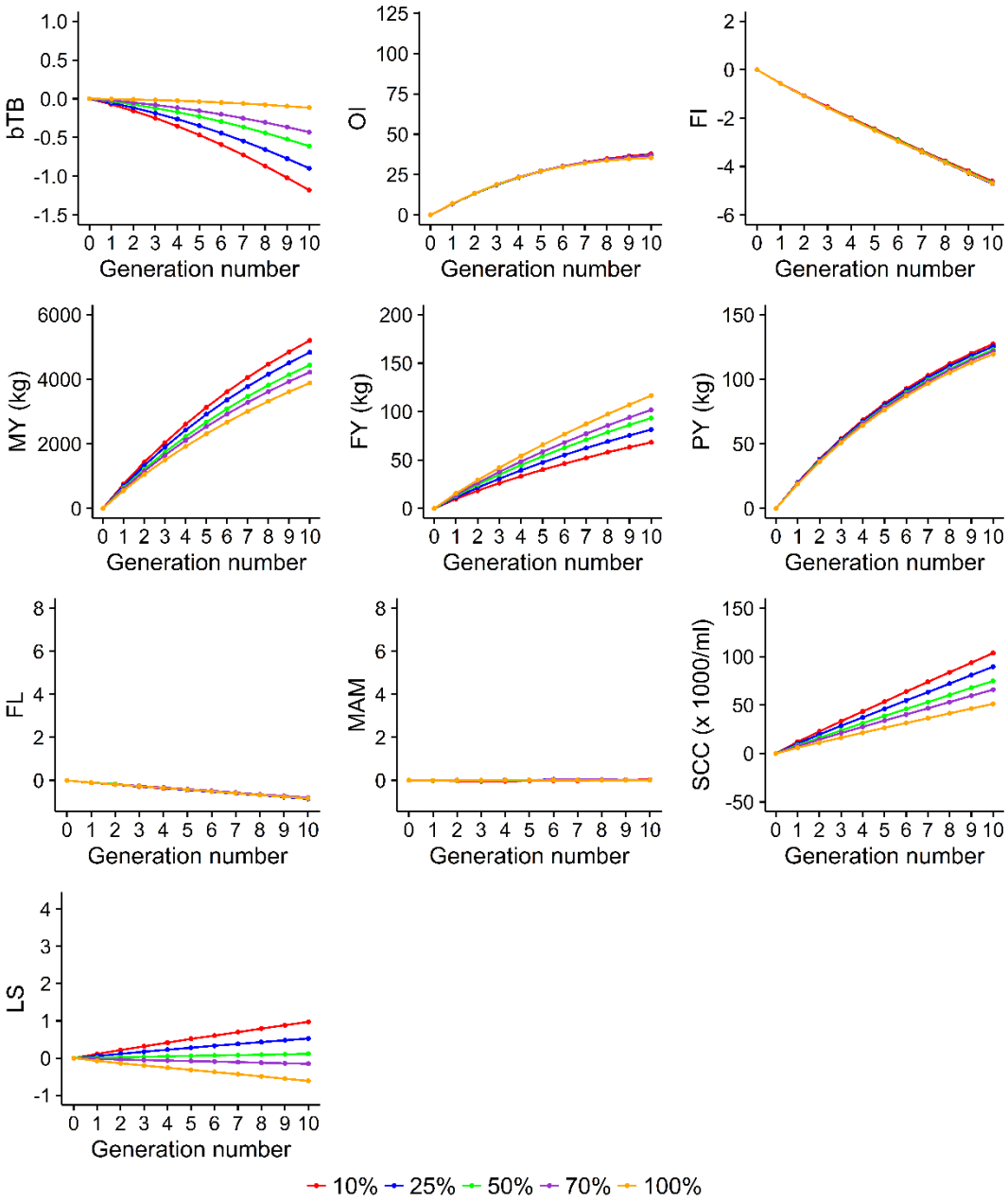

Figure 4.6 Genetic trends for susceptibility to bovine tuberculosis (bTB) and overall index (OI), fertility index (FI), milk yield (MY), milk fat yield (FY), milk protein yield (PY), lifespan (LS), feet and legs (FL), mammary (MAM) and milk somatic cell count (SCC). Selection intensities against bTB susceptibility correspond to selection of the 10, 25, 50, 70 and 100% (no selection) most resistant sires, followed by selection of the best 10% of remaining sires for increased PY. Vertical axes show the average genetic merit of all animals.

4.4 Discussion

In the present study, a stochastic simulation model of independent culling levels selection was used to determine the long-term effects of selection for bTB resistance on other economically important traits. Overall, results suggest that selecting for increased resistance to bTB will not have a major negative impact on other important traits, particularly when the breeding programme is underpinned by an overall selection index that optimally combines individual traits. In the UK, the overall index is the main tool with which the dairy industry selects the best performing sires [8]. This is consistent with the relatively high selection intensities for breeding goal traits considered in the present study.

Genetic trends presented for individual traits reflect selection based on true breeding values of animals, which assumes a perfect accuracy of the genetic evaluation. Therefore, these trends represent the upper limit of expected estimates. This reflects selection that assumes sires to have a high number of daughters to ensure highly accurate estimated breeding values. Usually, sires with highly accurate estimated breeding values are preferred including in the current selection criteria for bTB and other traits.

Results of the present study depend on the genetic correlations assumed between bTB and the other traits. These genetic correlation estimates were drawn from different previous studies in the literature and can be viewed as indicative of possible future impacts. Results demonstrate the potential cumulative benefits when correlations with bTB are favourable, as was the case with milk and protein yield in the present study. Thus, cows sired by bTB resistant bulls will not only be likely to be resistant but also produce more liquid milk and milk protein. This is in agreement with

a phenotypic study in ROI which reported that MY was significantly lower for bTB infected cows when compared to the non-infected ones [23]. This scenario would be applicable to breeding programmes in high bTB risk areas where intense selection for bTB resistance might be practiced without losing on improving milk production. Another study found no significant differences in milk production between healthy and bTB infected cattle [7] also supporting the notion that selection on bTB may not affect milk yield..

Results also demonstrate the potentially adverse effect of bTB selection on traits with an antagonistic correlation, as was the case with milk fat yield and SCC in the present study. Selection to reduce susceptibility to bTB slowed down progress on fat yield, although the effect was not major unless very intense selection was practiced on bTB. However, the latter is not expected to be the case in the first instance, as it is anticipated that bTB genetic evaluations will mainly be used to screen candidate sires and avoid those with very poor genetic merit for bTB resistance.

The current aim of improving the fertility of the national herd can be achieved without negative consequences from selecting animals that are resistant to bTB. However, care must be taken when improvement for SCC, which is an indicator of mastitis [24, 25] is sought. SCC should be monitored especially when high selection intensities for resistance to bTB are opted for, which in the present study lowered the response to selection for reduced SCC. Therefore, the aim should be to find a balance and prevent unfavourably high incidences of SCC (mastitis) while targeting resistance to bTB. Boland et al. [26] reported that, phenotypically, SCC in cows infected with bTB compared to SCC in non-infected cows was not significantly different in Irish dairy cattle. Similarly, the UK study of Banos et al. [2] reported that there was no

correlation between bTB and SCC EBVs. Thus, the moderate unfavourable correlation assumed in the present study might be indicative of the worst case scenario with regards to improving resistance to both bTB and mastitis. Mastitis remains one of the most important diseases in the dairy industry with similar consequences as bTB with regard to high costs incurred in disease management [27].

Based on assumptions in the present study, the concern is that when selection for resistance to bTB is coupled with selection for production traits, incidences of lameness or poor locomotion would likely increase in future animals. However, in practise this could be altered depending on the magnitude of real genetic correlations of bTB and conformation traits studied. Sound feet and legs are important especially in grazing systems where superior locomotion characteristics enable efficient grazing [28]. Equally important are improved mammary traits because they represent cows with better udder attachment, depth and efficient milking [28].

Usually, when independent culling levels selection is applied and unfavourable genetic correlations exist, it has been suggested that one selects for the most important trait in the first step since it gives more leverage to that particular trait [29]. Therefore, if the selection trend is for breeders to consider bTB resistance before other traits, that will likely favour response to selection for bTB resistance particularly with regards to unfavourably correlated traits.

Antagonistic and unfavourable relationships between susceptibility to bTB and other traits can be best managed by assimilating bTB genetic evaluations in the national overall index [30], or at least a sub-index suitable for geographic areas affected by the disease. In the current set-up, independent culling levels selection of bTB with other traits as simulated in this study may inflate the importance of bTB

relative to the other traits. Indeed, both performance and health traits influence profitability and hence should each be given optimal weighting. Selection index methodology becomes appealing in such cases because it is designed to weight traits by their economic merit [31]. Therefore, assimilation of bTB in the national selection index would avert problems of either under or over-estimating its economic importance in the greater scheme of things. However, adoption of resistance to bTB in the UK selection index should be preceded by estimation of genetic correlations among the individual traits from phenotypic data and fitting all traits simultaneously. This could be followed by a feasibility study.

Finally, the contribution of national breeding programmes to improve genetic merit for animal health traits has been well documented [32-34]. In the case of bTB, breeding companies may recommend only desirable bulls in terms of bTB resistance to high risk areas. Over time, this is expected to lead to a general reduction in bTB prevalence in the national herds especially in bTB endemic areas. Furthermore, infection rate may quickly be reduced because as animals become more resistant to bTB, their likelihood of being infected decreases hence possibly reducing overall herd infectivity. Considering the favourable genetic correlations of genetic evaluations for bTB resistance with longevity and overall index in the UK [2], it is possible that, while selecting sires with high overall index, the UK dairy industry could have been already indirectly selecting for improved resistance to bTB.

4.5 Conclusions

Results of the present study demonstrate that, generally, genetic selection for resistance to bTB is not likely to exact major adverse effects on other traits, particularly when genetic selection is based on an overall index carefully combining individual

traits. However, selection priorities and final decisions rest with the breeders themselves. For high risk areas where bTB is endemic, it might be reasonable and economically justifiable to first select sires resistant to bTB before considering other traits. This will assist farmers to leverage on the existing favourable genetic correlations between susceptibility to bTB with the overall selection index. The impact of genetic selection for increased resistance to bTB on other cow traits would be largely dictated by the respective genetic correlations. In the present study, genetic correlation estimates were drawn from different previous studies. An overhaul of these estimates using the UK national database would be warranted especially if bTB genetic evaluations were to be included in an overall selection index.

4.6 References

1. Richardson IW, Bradley DG, Higgins IM, More SJ, McClure J, Berry DP. Variance components for susceptibility to *Mycobacterium bovis* infection in dairy and beef cattle. Genet Sel Evol. 2014; 46(1):1.
2. Banos G, Winters M, Mrode R, Mitchell A, Bishop SC, Woolliams JA, Coffey MP. Genetic evaluation for bovine tuberculosis resistance in dairy cattle. J Dairy Sci. 2016; 100(2):1272-1281.
3. Brotherstone S, White I, Coffey M, Downs S, Mitchell A, Clifton-Hadley R, More S, Good M, Woolliams J. Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*. J Dairy Sci. 2010; 93(3):1234-1242.
4. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Brotherstone S, Berry DP. Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. J Dairy Sci. 2009; 92(7):3447-3456.

5. Tsairidou S, Woolliams JA, Allen AR, Skuce RA, McBride SH, Wright DM, et al. Genomic prediction for tuberculosis resistance in dairy cattle. PLoS One. 2014; 9(5):e96728.

6. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, et al. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity. 2014; 112(5):543-551.

7. Petukhov V, Kochnev N, Panov B, Korotkevich O, Kulikova S, Marenkov V. Genetics of cattle resistance to tuberculosis. In: Proc 6th World Congress on Genetics Applied to Livestock Production, Amidale, Australia, 11-16 January. 1998.

8. Agriculture and Horticulture Development Board (AHDB). Profitable lifetime index. https://dairy.ahdb.org.uk/technical-information/breeding-genetics/£pli/ (Accessed 21st August 2017).

9. Wall E, Brotherstone S, Woolliams J, Banos G, Coffey M. Genetic evaluation of fertility using direct and correlated traits. J Dairy Sci. 2003; 86(12):4093-4102.

10. Pryce JE, Brotherstone S. Estimation of lifespan breeding values in the UK and their relationship with health and fertility traits. Interbull Bulletin. 1999(21):166.

11. Pritchard T, Coffey M, Mrode R, Wall E. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal. 2013; 7(1):34-46.

12. Knaus W. Dairy cows trapped between performance demands and adaptability. J Sci Food Agric. 2009; 89(7):1107-1114.

13. Oltenacu PA, Algers B. Selection for increased production and the welfare of dairy cows: are new breeding goals needed? AMBIO: A Journal of the Human Environment. 2005; 34(4):311-315.

14. Pryce J, Veerkamp R, Thompson R, Hill W, Simm G. Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. Anim Sci. 1997; 65(3):353-360.

15. Gonda M, Chang Y, Shook G, Collins M, Kirkpatrick B. Effect of Mycobacterium paratuberculosis infection on production, reproduction, and health traits in US Holsteins. Prev Vet Med. 2007; 80(2):103-119.

16. Heuer C, Healy A, Zerbini C. Economic effects of exposure to bovine viral diarrhea virus on dairy herds in New Zealand. J Dairy Sci. 2007; 90(12):5428-5438.

17. Heringstad B, Chang Y, Gianola D, Klemetsdal G. Genetic association between susceptibility to clinical mastitis and protein yield in Norwegian dairy cattle. J Dairy Sci. 2005; 88(4):1509-1514.

18. Lawes J, Harris K, Brouwer A, Broughan J, Smith N, Upton P. Bovine TB surveillance in Great Britain in 2014. Vet Rec. 2016; 178(13):310-315.

19. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Berry DP. Genetic correlations between measures of Mycobacterium bovis infection and economically important traits in Irish Holstein-Friesian dairy cows. J Dairy Sci. 2010; 93(11):5413-5422.

20. Interbull. National genetic evaluation forms provided by countries. http://interbull.org/ib/geforms (Accessed 11th September 2017).

21. Mrode RA. Linear models for the prediction of animal breeding values: CAB International; 2014.

22. Pryce J, Coffey M, Brotherstone S. The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. J Dairy Sci. 2000; 83(11):2664-2671.

23. Boland F, Kelly G, Good M, More S. Bovine tuberculosis and milk production in infected dairy herds in Ireland. Prev Vet Med. 2010; 93(2):153-161.

24. Rupp R, Boichard D. Genetics of resistance to mastitis in dairy cattle. Vet Res. 2003; 34(5):671-688.

25. Philipsson J, Ral G, Berglund B. Somatic cell count as a selection criterion for mastitis resistance in dairy cattle. Livest Prod Sci. 1995; 41(3):195-200.

26. Boland F, Kelly G, Good M, More S. Bovine tuberculosis and udder health in Irish dairy herds. Vet J. 2012; 192(1):71-74.

27. Halasa T, Huijps K, Østerås O, Hogeveen H. Economic effects of bovine mastitis and mastitis management: a review. Vet Q. 2007; 29(1):18-31.

28. Berry DP, Buckley F, Dillon P, Evans RD, Veerkamp RF. Genetic relationships among linear type traits, milk yield, body weight, fertility and somatic cell count in primiparous dairy cows. Irish J Agr Food Res. 2004:161-176.

29. Pesek J, Baker R. Comparison of tandem and index selection in the modified pedigree method of breeding self-pollinated species. Can J Plant Sci. 1969; 49(6):773-781.

30. Philipson J, Banos G, Arnason T. Present and future uses of selection index methodology in dairy cattle. J Dairy Sci. 1994; 77(10):3252-3261.

31. Cameron N. Selection indices and prediction of genetic merit in animal breeding: CAB International; 1997.

32. Heringstad B, Klemetsdal G, Ruane J. Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. Livest Prod Sci. 2000; 64(2):95-106.

33. Heringstad B, Klemetsdal G, Ruane J. Responses to selection against clinical mastitis in the Norwegian cattle population. Acta Agric Scand Sect A Anim Sci. 2001; 51(1):15-20.

34. Berry DP, Bermingham ML, Good M, More SJ. Genetics of animal health and disease in cattle. Irish Vet J. 2011; 64(1):5.

CHAPTER 5

General Discussion

5.1 Scope of thesis

The objectives of this PhD thesis were to contribute to the study of the genetic architecture of cattle susceptibility to bovine tuberculosis (bTB) and assess the impact of genetic selection for increased resistance to bTB on the disease dynamics and the breeding programme. The former included identification and analysis of individual SNPs and genomic regions underlying host genetic variation with regards to bTB infection. For the assessment and quantification of the impact of long-term genetic selection for increased resistance to bTB on disease prevalence and dynamics, simulations based on a genetic epidemiological model were performed to mimic selection in a heterogeneous population. The genetic consequences of selecting animals for increased resistance to bTB on other economically important dairy cattle traits were also examined with simulation studies.

There were several novel aspects of study in the present thesis. Firstly, the genomic background of novel bTB phenotypes was studied in genome-wide association studies (GWAS), regional heritability mapping (RHM) and chromosomal association analysis. Such analyses had not been conducted on the British Holstein cattle population before. Trait definitions included: a) confirmed cases, b) skin test response regardless of post-mortem examination results and all cases from b plus a rare cohort of animals which were either skin test negative or inconclusive but following slaughter, were diagnosed with bTB lesions during post-mortem

examination [1]. The latter has been decided as the phenotype of choice in the official UK national genetic evaluation for bTB. Sire de-regressed estimated breeding values (EBVs) were used, constituting robust phenotypes based on an aggregate of disease incidence from a large number of daughters. Furthermore, phenotypes analysed in the present study represent a wide range of possible bTB outcomes with regards to the current testing regime in the UK.

Secondly, there is novelty in the genetic epidemiological model developed in the present study. The model provides the first assessment of the impact of selection for increased bTB resistance on the disease dynamics and future prevalence. Development of this bTB model differs from previous epidemiological models [2-9] in two main ways: 1) It adds the all-too important dimension of accounting for genetic variation in host susceptibility to bTB, which has been neglected in previous studies. Previous bTB modelling studies tended to emphasise on between herd transmissions rather than decomposing infection at an individual animal level. 2) Apart from accounting for host genetic variation, the model also explicitly explores another perspective in transmission dynamics of bTB which has not previously been considered. Thus, the assumption in the present study model that animals can be infectious before they can be detected by the skin test is a possibility [10, 11] that has not been previously considered despite the latency stage of bTB being poorly understood [12]. In addition, the robustness and relevance of the model in the present study emanate from the parameterisation of simulation outputs based on real bTB surveillance data from breakdowns involving a large number of cows (~1.2 million).

Thirdly, the current study presents for the first time predictions of the consequences of selecting for increased bTB resistance on other economically important dairy traits that are included in the genetic improvement programme.

5.2 Key results and contribution to current knowledge

Outcomes of this thesis established that there is a genetic component underlying susceptibility to bTB in the dairy cattle population that was studied. Results suggest that bTB, as defined here, is a moderately heritable and likely polygenic trait. Therefore, bTB is probably controlled by a large number of variants each of small effect size distributed across the entire genome. Putative quantitative trait loci (QTL) underlying susceptibility to bTB were identified on *Bos taurus* autosome (BTA) 2 and BTA 23. Plausible candidate genes identified on BTA 2 have been previously reported to be involved in prevention of disease progression in patients affected with HIV/AIDS [13] while the gene on BTA 23 was associated with immune response in humans [14]. Like HIV infection, bTB is a chronic and inflammatory illness and the host may engage some similar mechanisms to fight against infection.

This thesis adds to a growing body of literature which supports the concept to breed cattle for enhanced resistance to bTB [1, 15-19]. Although moderate estimates of heritability for resistance/susceptibility to bTB were obtained, these were generally higher than those reported in literature [15-20].

Genomic association results in the present study adds to the growing evidence that BTA 23 possibly plays a major role in dairy cattle susceptibility to bTB as other studies also identified QTLs on BTA 23 albeit at different regions [21, 22]. In more general terms, genomic association analyses in the present and previous studies [20-24] have identified different sets of markers and genomic regions linked with bTB

phenotypes, suggesting that resistance to infection is likely a trait following a polygenic inheritance mode.

When the phenotype analysed in the present study involved confirmed cases only, a lower heritability was obtained and different genomic regions were identified compared to the other two phenotypes. Furthermore, EBVs for this first phenotype had a lower correlation with those of the other two phenotypes. However, EBVs for the latter phenotypes were highly correlated to each other indicating that they were practically the same trait. This finding is in concordance with findings of Banos et al. [1]. While confirmed cases and skin test response in the present study were both indicative of infection with *Mycobacterium bovis*, they are likely to be different phenotypes as the latter is affected by the imperfection (incomplete sensitivity and specificity) of the skin test [25]. Furthermore, some animals do not show visible lesions in the abattoir (non-visible lesion reactors) because they are in the early stage of infection or are infected by other environmental *Mycobacterium* antigens that cross-react with *M. bovis* antigens used in the skin test [25]. However, despite the differences between the two traits, the moderate positive genetic correlation (0.54) in the present study suggests that to an extent both phenotypes were under control of similar genes. Therefore, selection for either trait will improve the other.

Results from the present study also highlighted the utility of RHM in identification of significant associations where GWAS could have limitations [26]. RHM identified additional regions associated with susceptibility to bTB apart from those that were identified by GWAS. RHM revealed the collective significant effect of neighbouring SNPs, whose individual effects were too small to be detected by GWAS. In addition, the suggestive QTL on BTA 23 under GWAS reached genome-

wide significance in the RHM analysis. RHM was used to confirm GWAS results in other bTB studies of Bermingham et al. [20] and Wilkinson et al. [22]. In the former study, while RHM confirmed GWAS results it did not identify any new regions associated with bTB resistance. However, in the latter study bTB cases from the former [20] were dissected into visible lesion and non-visible lesion phenotypes. The latter study [22] confirmed GWAS results from the former [20] and identified new regions associated with bTB. These findings in conjunction with those from the present study indicate the influence of trait definition in identification of QTL within and between populations.

Simulation of genetic selection for reduced susceptibility to bTB revealed that selection was a viable option in reducing the prevalence and severity of the disease. This would be achieved by simultaneous reduction in the bTB underlying susceptibility level in the population, decrease of the probability that a breakdown occurs, and a mitigated severity in the breakdowns that do eventually occur. Such an approach could complement current non-genetic control strategies. The advantage of genetic selection over conventional control strategies is that the effects of the former are cumulative and permanent [27]. Findings from this study concur with those of other studies which found that genetic selection reduced prevalence, risk and severity of epidemics in various diseases of livestock and fish [28-32]. Furthermore, results confirmed that, in general, moderate selection for reduced susceptibility to bTB would not adversely affect other economically important traits.

Due to the novel design of the genetic epidemiological model developed in the present study, it was a challenge to directly compare obtained epidemiological parameter estimates with those of previous studies because a different state chronology

in bTB progression was assumed, as mentioned above. Nonetheless, the transmission coefficient β in the present study fell within the range of previous bTB model estimates [2-9]. The transmission coefficient is an important epidemiological parameter because it describes the contact rate between animals and the probability of the contact resulting in a successful transmission [33]. The length of exposed stage in the current study ($E \rightarrow I$) and that from the recent epidemiological study in the UK [6] ($E \rightarrow T$) were almost similar despite representing different order of events in bTB transmission. The duration between an infectious animal becoming test-sensitive in the present study was relatively short (2days). This short interval was also realised in another study model assuming animals in the E state were infectious before being test-sensitive [8]. These findings generally suggest that for bTB, once animals become infectious, they do not take long before they become detectable.

In the following sections, the practical application and implication of results from the present study are discussed along with challenges and opportunities, as well as possible future research.

5.3 Implication and practical application of results

Breeding for host resistance to bTB provides a complementary strategy to mainstream control interventions, which have so far been inadequate in containing the disease. The strong impression from the current study is that bTB is a polygenic trait and this notion is supported by the absence of common QTLs with large effects across different genomic association studies [20-24]. In such circumstances, infinitesimal models that target variants across the entire genome may be preferred to selection based on individual loci [34].

In practice, selective breeding for disease resistance is possible and has been achieved in several livestock species, including in sheep and goats resistance to internal parasites [35, 36] and mastitis in dairy cattle [37]. An important aspect of selection is estimation of accurate EBVs for the trait of interest which can be used to select the best animals for breeding. In the UK, EBVs for susceptibility to bTB based on phenotypic and pedigree data [1] are available to the dairy industry and bTB has been added to the national genetic evaluation programme. The genetic epidemiological model developed in the present thesis built on the existence of such selection tools and also simulated a wide range of scenarios exploring different selection intensities placed on bTB resistance. Considering that bTB occurs at different prevalence levels across regions [38], farmers in high risk areas may desire to more strongly select against bTB than their counterparts in low risk areas.

Epidemiological models can play an integral part in decision making during disease epidemics [39, 40]. In the present study, it was demonstrated that genetic selection would significantly reduce bTB prevalence but would unlikely eradicate the disease within the set time for England and Wales (by year 2038) without additional interventions. To decision makers, this information makes it worthwhile to consider selection alongside other interventions including existing ones to expedite the eradication process. Genetic selection drastically reduces secondary cases and possibly R_0 especially during the first few generations, but to accelerate the eradication process including other interventions is important. Continued emphasis on dealing with external sources of infection such as wildlife, transmission between neighbouring herds and movement restrictions in affected herds is necessary. In the epidemiological model developed in the present study, these alternative sources of infection are

represented by the parameter α and improving these factors will reduce α. While α is kept constant in the present study, in reality this parameter is expected to decline across generations as genetic selection is likely to reduce transmission between wildlife and cattle and between cattle. This parameter is also expected to vary across regions depending on bTB prevalence and density of wildlife, e.g. badgers in UK which are the common species that transmit bTB. Furthermore, improving sensitivity of bTB diagnostic tools and reducing testing intervals (particularly in areas of high prevalence) may also accelerate the eradication process by increasing the rate of detection of infected animals. Detection and removal of more infected animals will reduce the overall herd infectivity. Other options to explore could include 1) selecting for increased resistance in cow dams in addition to sires in order to increase response to selection. This should be practical considering the relatively good data structure for dairy breeds in the country and 2) genetic selection to reduce infectivity in addition to susceptibility [41].

However, as shown in the present thesis, conventional selection for resistance to bTB is likely to be a long process and possible challenges may emerge along the way. For example, derivation of EBVs would mean continued collection of phenotypic data and this would require a population to be undergoing an epidemic with equal probability of exposure to infection of all contemporaneously kept animals [42]. However, as resistance in the national herd improves and bTB prevalence declines probably with contribution of selection, identifying bTB cases based on phenotypes may become more challenging. This would be exacerbated by incomplete sensitivity of the skin test (main diagnostic test), which has the propensity to miss infected animals [25].

When such a problem arises, genomic selection based on genomic EBVs (GEBVs) becomes worth undertaking. GEBVs for bTB [43] are already available to the UK dairy industry. The other advantage with genomic selection is that farmers would be able to identify and exclude young animals at an early age if they were predicted to be susceptible to bTB. Consequently, genomic selection has the potential to considerably reduce generation interval thereby increasing genetic gains and response to selection [44, 45]. This presents an even more potent strategy to achieve the goal of bTB free status by year 2038 in England and Wales. Identification of the worst animals based on their GEBVs and their removal before they have the opportunity to infect others in the herd would not only reduce the number of susceptible individuals but will also reduce the overall herd infectivity over time. Likewise, the potential of a herd to transmit infection to wildlife reservoirs (such as the Eurasian badger in the UK) and back to cattle is likely to also decline.

Unlike conventional EBVs, GEBVs can be estimated in the absence of bTB phenotypes, provided sufficient phenotypic and genotypic information is available for the reference population [17]. Once the marker effects from the reference population data are estimated, they can be used to predict GEBVs for young animals with genotypes but no phenotypes. Since linkage disequilibrium patterns between markers and the actual QTLs may change due to selection, periodic re-estimation of the marker effects may be required [46]. GEBVs are usually computed as a function of individual markers [17] based on genetic models similar to those used in GWAS in the present study. Therefore, application of genomic selection for reduced susceptibility to bTB [43] in the dairy cattle population studied in the present study is possible. However, in many breeding programmes there are challenges of having a small proportion of

animals with appropriate phenotypes being genotyped. In such cases, methodology such as single step approach could be considered in the genetic evaluation due to its ability to effectively combine genotyped and non-genotyped animals and linking them through both genomic and pedigree relationships [47]. This approach could improve prediction accuracies in bTB genetic evaluations.

Identifying genomic regions and SNPs associated with a trait of interest could inform more accurate calculation of GEBVs that places more emphasis on markers and/or genomic regions with larger effects. For example, in the case of infectious pancreatic necrosis in Atlantic salmon where a particular genomic region accounted for a substantial amount of variation in disease resistance [48]. Computation of GEBVs with models placing more emphasis on SNPs and genomic regions with large effects on the trait can be achieved using Bayesian approaches [49, 50]. In this context, genomic regions identified in the present study combined with regions revealed in previous studies might be used to inform more accurate derivation of GEBVs for bTB resistance. The same information might also contribute to the development of a target-specific bTB SNP array. This could be facilitated by the emergence of next-generation sequencing technologies which provides an opportunity not only for large scale SNP discovery but also faster and more cost effective solution to genotyping based on novel target-specific arrays [51, 52]. Target specific arrays for bTB could probably increase the resolution of relevant pathways to bTB susceptibility.

Furthermore, results from the present study could provide data for functional analyses including expression and pathways analyses [53], as well as post-functional analysis eventually leading to the identification of causal mutations controlling bTB susceptibility. Identification of novel biochemical pathways involved in host response

to *Mycobacterium bovis* may inform future efforts to produce better disease diagnostic tools and vaccines.

Moreover, with advances in relevant technology, identified QTLs could provide potential targets for genome editing in advanced breeding programmes that seek to enhance livestock resistance to diseases, especially if the QTL effects are of concern.

Finally, putative genetic markers revealed in the present and other genomic studies may be used to facilitate marker assisted selection, whereby genetically susceptible sires are identified and removed from breeding, especially in high risk areas of bTB. While this might be viewed as insufficient in the case of a likely polygenic trait like bTB, Cox et al. [54] have demonstrated that in situations where the basic reproductive ratio (R_0) is slightly greater than 1 as was the case in their bTB study (R_0 = 1.02 - 1.11) even a modest reduction in infection between animals could still significantly reduce the observed bTB epidemics over time. However, GEBVs would still be much more accurate than single SNPs at identifying and removing genetically susceptible sires.

Before implementation of a breeding programme to select for disease resistance there are several issues that warrant attention. The desirability of breeding for disease resistance depends upon whether there are compromises with other economically important traits [55]. Results from this thesis indicate that it is possible to breed for resistance with minimal consequences on other traits. However, such breeding programmes should consider using bTB genetic evaluations within an overall selection index, in the first instance targeting high risk areas. The advantage with the

overall index is that trait combinations are optimised through economic weights. As a result the possibly unfavourable correlation between bTB and certain traits can be managed better.

5.4 Future considerations

5.4.1 Further bovine tuberculosis and meta-analysis studies

Generally, application of genomic association results is often challenged by the likelihood that the findings may be more directly relevant to the population for which they were derived. In the case of bTB, there have been inconsistent results regarding QTLs identified in different populations [20-24]. Consequently, the search for common QTLs continues. Therefore, further genomic studies of different populations and breeds would be necessary. Alternatively, bTB data across different populations could be consolidated especially as more data becomes available. These data could then be used in meta-analysis studies [24] which may increase power to detect QTL. Results from this PhD add to the existing information regarding bTB at both individual and population level.

5.4.2 Exploration with the genetic epidemiological model

The genetic epidemiological model developed in this thesis offers flexibility and therefore could be extended to accommodate additional factors which may be relevant to bTB dynamics. These could be alternative diagnostic testing protocols such as post-mortem examination and gamma-interferon assays, and demographic characteristics such as births, deaths and age of animal. Outside bTB, the concept of accounting for genetic variation in the analysis of disease transmission and epidemiology as presented in this PhD thesis can be applied to other infectious diseases of livestock especially

with similar transmission patterns. This would entail further development of models that appropriately capture the dynamics of the specific diseases.

5.4.3 Selective breeding on additional bovine tuberculosis phenotypes

In the case of bTB, the definition of the susceptibility phenotype has been the main focus of many studies [15-20] with little attention being paid to other potentially important phenotypes related with infectivity and tolerance to disease. Infectivity is defined as an individual's ability to transmit infection [56, 57]. The genetic epidemiological model developed in the present study assumed the same infectivity across individual animals. However, there is evidence for phenotypic variation in infectivity, which can be manifested by the existence of super-spreaders [6, 58]. Furthermore, a theoretical study based on a generic disease model demonstrated that selection on both susceptibility and infectivity may reduce disease prevalence and severity faster than selection based only on disease susceptibility [41]. The key challenge that lies ahead is the definition of infectivity phenotypes from field data. Unlike susceptibility, infectivity is a trait expressed through social interactions among animals affecting the health status of group members rather than the individual animal expressing the phenotype. There are theoretical algorithms to assess infectivity based on statistical inference and probability [57, 59, 60] that may be adapted to address the issue in conditions of bTB infection. The next challenge would then be to characterise the genetic background of infectivity including heritability estimates and genetic correlation with susceptibility. These parameters would be necessary in order to incorporate bTB infectivity in the breeding programme.

The other possible trait which has not been studied before with regard to bTB is tolerance to disease. Tolerance is the ability of a host to maintain fitness or performance despite infection with a pathogen [61]. However, in the case of bTB where the ultimate goal is to eradicate the disease, breeding for resistance makes sense because an increase in tolerance would not necessarily constrain pathogen replication and, therefore, would not eliminate the disease [62].

5.4.4 Assimilation of bovine tuberculosis in the overall index

Although the benefits of including bTB in the current breeding programme in the UK could reduce financial requirements to control the disease, there is a need to balance such a decision against costs. There is a possibility that considering bTB selection first and the other traits afterwards in an independent culling levels selection scheme might overestimate the importance of the former relative to the latter. Therefore the optimal way would be to include bTB in the selection index following appropriate weighting [63]. This should entail estimation of updated genetic correlations among traits and a feasibility study similar to those undertaken before incorporating fertility [64] and lameness and mastitis [65] into national selection indices.

5.5 General conclusions

The results reported in this PhD thesis suggest that selection for improved bTB resistance in cattle can be both feasible and effective in reducing prevalence of bTB in the national herd. While the majority of previous genetic studies have focused on dairy cattle, the main principles can also be applicable to beef cattle, which have so far received little attention in this regard. Finally, any efforts aimed to control and

eradicate bTB should consider genetic selection as an additional complementary approach that should be applied alongside the pool of existing measures.

5.6 References

1. Banos G, Winters M, Mrode R, Mitchell A, Bishop SC, Woolliams JA, Coffey MP. Genetic evaluation for bovine tuberculosis resistance in dairy cattle. J Dairy Sci. 2016; 100(2):1272-1281.
2. Biek R, O'Hare A, Wright D, Mallon T, McCormick C, Orton RJ, et al. Whole genome sequencing reveals local transmission patterns of Mycobacterium bovis in sympatric cattle and badger populations. PLoS Pathog. 2012; 8(11):e1003008.
3. Bekara MEA, Courcoul A, Benet J-J, Durand B. Modeling tuberculosis dynamics, detection and control in cattle herds. PLoS One. 2014; 9(9):e108584.
4. Fischer E, Van Roermund H, Hemerik L, Van Asseldonk M, De Jong M. Evaluation of surveillance strategies for bovine tuberculosis (Mycobacterium bovis) using an individual based epidemiological model. Prev Vet Med. 2005; 67(4):283-301.
5. Brooks-Pollock E, Roberts GO, Keeling MJ. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature. 2014; 511(7508):228-231.
6. O'Hare A, Orton RJ, Bessell PR, Kao RR. Estimating epidemiological parameters for bovine tuberculosis in British cattle using a Bayesian partial-likelihood approach. Proc R Soc Lond B Biol Sci. 2014; 281(1783).

7. Perez AM, Ward MP, Charmandarián A, Ritacco V. Simulation model of within-herd transmission of bovine tuberculosis in Argentine dairy herds. Prev Vet Med. 2002; 54(4):361-372.

8. Conlan AJ, McKinley TJ, Karolemeas K, Pollock EB, Goodchild AV, Mitchell AP, Birch CP, Clifton-Hadley RS, Wood JL. Estimating the hidden burden of bovine tuberculosis in Great Britain. PLoS Comp Biol. 2012; 8(10):e1002730.

9. Barlow N, Kean J, Hickling G, Livingstone P, Robson A. A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Prev Vet Med. 1997; 32(1-2):57-75.

10. Neill S, Hanna J, O'brien J, McCracken R. Excretion of Mycobacterium bovis by experimentally infected cattle. Vet Rec. 1988; 123(13):340-343.

11. McIlroy S, Neill S, McCracken R. Pulmonary lesions and Mycobacterium bovis excretion from the respiratory tract of tuberculin reacting cattle. Vet Rec. 1986; 118(26):718-721.

12. Jones GJ, Pirson C, Gideon HP, Wilkinson KA, Sherman DR, Wilkinson RJ, Hewinson RG, Vordermeier HM. Immune responses to the enduring hypoxic response antigen Rv0188 are preferentially detected in Mycobacterium bovis infected cattle with low pathology. PLoS One. 2011; 6(6):e21371.

13. Troyer JL, Nelson GW, Lautenberger JA, Chinn L, McIntosh C, Johnson RC, et al. Genome-wide association study implicates PARD3B-based AIDS restriction. J Infect Dis. 2011; 203(10):1491-1502.

14. Baek S-H, Huang B, Chang HW. RNF144b is a negative regulator in TLR2-mediated NF-kB activation. J Immunol. 2016; 196(1):132.134.

15. Bermingham ML, More SJ, Good M, Cromie AR, Higgins IM, Brotherstone S, Berry DP. Genetics of tuberculosis in Irish Holstein-Friesian dairy herds. J Dairy Sci. 2009; 92(7):3447-3456.

16. Brotherstone S, White I, Coffey M, Downs S, Mitchell A, Clifton-Hadley R, More S, Good M, Woolliams J. Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*. J Dairy Sci. 2010; 93(3):1234-1242.

17. Tsairidou S, Woolliams JA, Allen AR, Skuce RA, McBride SH, Wright DM, et al. Genomic prediction for tuberculosis resistance in dairy cattle. PLoS One. 2014; 9(5):e96728.

18. Richardson IW, Bradley DG, Higgins IM, More SJ, McClure J, Berry DP. Variance components for susceptibility to *Mycobacterium bovis* infection in dairy and beef cattle. Genet Sel Evol. 2014; 46(1):1.

19. Petukhov V, Kochnev N, Panov B, Korotkevich O, Kulikova S, Marenkov V. Genetics of cattle resistance to tuberculosis. In: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Amidale, Australia, 11-16 January. 1998.

20. Bermingham ML, Bishop SC, Woolliams JA, Pong-Wong R, Allen AR, McBride SH, et al. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity. 2014; 112(5):543-551.

21. Richardson IW, Berry DP, Wiencko HL, Higgins IM, More SJ, McClure J, Lynn DJ, Bradley DG. A genome-wide association study for genetic susceptibility to *Mycobacterium bovis* infection in dairy cattle identifies a susceptibility QTL on chromosome 23. Genet Sel Evol. 2016; 48(1):1.

22. Wilkinson S, Bishop S, Allen A, McBride S, Skuce R, Bermingham M, Woolliams J, Glass E. Fine-mapping host genetic variation underlying outcomes to Mycobacterium bovis infection in dairy cows. BMC Genomics. 2017; 18(1):477.

23. Finlay EK, Berry DP, Wickham B, Gormley EP, Bradley DG. A genome wide association scan of bovine tuberculosis susceptibility in Holstein-Friesian dairy cattle. PLoS One. 2012; 7(2):e30545.

24. Tsairidou S, Woolliams J, Allen A, Skuce R, McBride S, Pong-Wong R, et al. A meta-analysis for bovine tuberculosis resistance in dairy cattle. In. Proceedings of the British Society of Animal Science, Chester, UK, 14-15 April; 2015.

25. De la Rua-Domenech R, Goodchild AT, Vordermeier HM, Hewinson RG, Christiansen KH, Clifton-Hadley RS. Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Res Vet Sci. 2006; 81(2):190-210.

26. Nagamine Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Rudan I, et al. Localising loci underlying complex trait variation using regional genomic relationship mapping. PLoS One. 2012; 7(10):e46501.

27. Falconer DS. Introduction to quantitative genetics, 4th edn. Harlow, UK: Pearson Education Limited; 1996.

28. MacKenzie K, Bishop SC. Utilizing stochastic genetic epidemiological models to quantify the impact of selection for resistance to infectious diseases in domestic livestock. J Anim Sci. 2001; 79(8):2057-2065.

29. Gharbi K, Matthews L, Bron J, Roberts R, Tinch A, Stear M. The control of sea lice in Atlantic salmon by selective breeding. J R Soc Interface. 2015; 12(110):20150574.

30. Nieuwhof GJ, Conington J, Bishop SC. A genetic epidemiological model to describe resistance to an endemic bacterial disease in livestock: application to footrot in sheep. Genet Sel Evol. 2009; 41(1):19.

31. Lipschutz-Powell D, Woolliams JA, Bijma P, Pong-Wong R, Bermingham ML, Doeschl-Wilson AB. Bias, accuracy, and impact of indirect genetic effects in infectious diseases. Front Genet. 2012; 3.

32. MacKenzie K, Bishop S. A discrete-time epidemiological model to quantify selection for disease resistance. Anim Sci. 1999; 69(3):543-551.

33. Keeling MJ, Rohani P. Modeling infectious diseases in humans and animals: Princeton University Press, New Jersey, USA; 2008.

34. Bishop SC, Woolliams JA. Genomics and disease resistance studies in livestock. Livestock Science. 2014; 166:190-198.

35. Bishop S, Morris C. Genetics of disease resistance in sheep and goats. Small ruminant research. 2007; 70(1):48-59.

36. Kemper K, Palmer D, Liu S, Greeff J, Bishop S, Karlsson L. Reduction of faecal worm egg count, worm numbers and worm fecundity in sheep selected for worm resistance following artificial infection with Teladorsagia circumcincta and Trichostrongylus colubriformis. Vet Parasitol. 2010; 171(3):238-246.

37. Heringstad B, Klemetsdal G, Ruane J. Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. Livest Prod Sci. 2000; 64(2):95-106.

38. Abernethy DA, Upton P, Higgins IM, McGrath G, Goodchild AV, Rolfe SJ, et al. Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995-2010. Vet Rec. 2013; 172(12):312.

39. Keeling MJ, Woolhouse ME, Shaw DJ, Matthews L, Chase-Topping M, Haydon DT, et al. Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogeneous landscape. Science. 2001; 294(5543):813-817.

40. Ferguson NM, Donnelly CA, Anderson RM. The foot-and-mouth epidemic in Great Britain: Pattern of spread and impact of interventions. Science. 2001; 292(5519):1155-1160.

41. Tsairidou S, Anacleto O, Raphaka K, Sanchez-Molano E, Banos G, Woolliams JA, Doeschl-Wilson AB. Enhancing genetic disease control by selecting for lower host infectivity In: 11th World Congress on Genetics Applied to Livestock Production, Auckland, New Zealand, 11-16 February. 2018.

42. Bishop SC, Woolliams JA. On the genetic interpretation of disease data. PLoS One. 2010; 5(1):e8940.

43. Mrode R. Application of various models for the genomic evaluation of bovine tuberculosis in dairy cattle. In: Proceedings of the 2016 Interbull Annual Meeting, Puerto Varas, Chile. 2016.

44. Hayes BJ, Bowman PJ, Chamberlain A, Goddard M. Invited review: Genomic selection in dairy cattle: progress and challenges. J Dairy Sci. 2009; 92(2):433-443.

45. Schaeffer L. Strategy for applying genome-wide selection in dairy cattle. J Anim Breed Genet. 2006; 123(4):218-223.

46. Solberg TR, Sonesson AK, Woolliams JA, Ødegard J, Meuwissen TH. Persistence of accuracy of genome-wide breeding values over generations when including a polygenic effect. Genet Sel Evol. 2009; 41(1):53.

47. Aguilar I, Misztal I, Johnson D, Legarra A, Tsuruta S, Lawlor T. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J Dairy Sci. 2010; 93(2):743-752.

48. Houston RD, Haley CS, Hamilton A, Guy DR, Mota-Velasco JC, Gheyas AA, et al. The susceptibility of Atlantic salmon fry to freshwater infectious pancreatic necrosis is largely explained by a major QTL. Heredity. 2010; 105(3):318-327.

49. Wang C-L, Ma P-P, Zhang Z, Ding X-D, Liu J-F, Fu W-X, Weng Z-Q, Zhang Q. Comparison of five methods for genomic breeding value estimation for the common dataset of the 15 th QTL-MAS Workshop. In: BMC proceedings. 2012. BioMed Central. S13.

50. Zeng J, Pszczola M, Wolc A, Strabel T, Fernando RL, Garrick DJ, Dekkers JC. Genomic breeding value prediction and QTL mapping of QTLMAS2011 data using Bayesian and GBLUP methods. In: BMC proceedings. 2012. BioMed Central. S7.

51. Reis-Filho JS. Next-generation sequencing. Breast Cancer Research. 2009; 11(3):S12.

52. Schuster SC. Next-generation sequencing transforms today's biology. Nat Methods. 2008; 5(1):16-18.

53. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2008; 37(1):1-13.

54. Cox D, Donnelly CA, Bourne FJ, Gettinby G, McInerney JP, Morrison WI, Woodroffe R. Simple model for tuberculosis in cattle and badgers. Proc Natl Acad Sci USA. 2005; 102(49):17588-17593.

55. Stear M, Bishop S, Mallard B, Raadsma H. The sustainability, feasibility and desirability of breeding livestock for disease resistance. Res Vet Sci. 2001; 71(1):1-7.

56. Lipschutz-Powell D, Woolliams JA, Doeschl-Wilson AB. A unifying theory for genetic epidemiological analysis of binary disease data. Genet Sel Evol. 2014; 46(1):1-12.

57. Anche MT, Bijma P, De Jong MC. Genetic analysis of infectious diseases: estimating gene effects for susceptibility and infectivity. Genet Sel Evol. 2015; 47(1):85.

58. Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. Superspreading and the effect of individual variation on disease emergence. Nature. 2005; 438(7066):355-359.

59. Anacleto O, Garcia-Cortés LA, Lipschutz-Powell D, Woolliams JA, Doeschl-Wilson AB. A novel statistical model to estimate host genetic effects affecting disease transmission. Genetics. 2015; 201(3):871-884.

60. Biemans F, Jong MC, Bijma P. A model to estimate effects of SNPs on host susceptibility and infectivity for an endemic infectious disease. Genet Sel Evol. 2017; 49(1):53.

61. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. Science. 2012; 335(6071):936-941.

62. Roy B, Kirchner J. Evolutionary dynamics of pathogen resistance and tolerance. Evolution. 2000; 54(1):51-63.

63. Cameron N. Selection indices and prediction of genetic merit in animal breeding: CAB international; 1997.

64. Pryce J, Veerkamp R. The incorporation of fertility indices in genetic improvement programmes. BSAS occasional publication. 2001:237-250.

65. Stott A, Coffey M, Brotherstone S. Including lameness and mastitis in a profit index for dairy cattle. Anim Sci. 2005; 80(1):41-52.